

(19)



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(11)

EP 0 757 058 A1

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
05.02.1997 Bulletin 1997/06

(51) Int Cl.⁶: **C07K 7/56, A61K 38/12**

(21) Application number: **96305345.9**

(22) Date of filing: **22.07.1996**

(84) Designated Contracting States:
**AT BE CH DE DK ES FI FR GB GR IE IT LI LU NL
PT SE**
Designated Extension States:
AL LT LV SI

(72) Inventor: **Rodriguez, Michael John**
Indianapolis, Indiana 46260 (US)

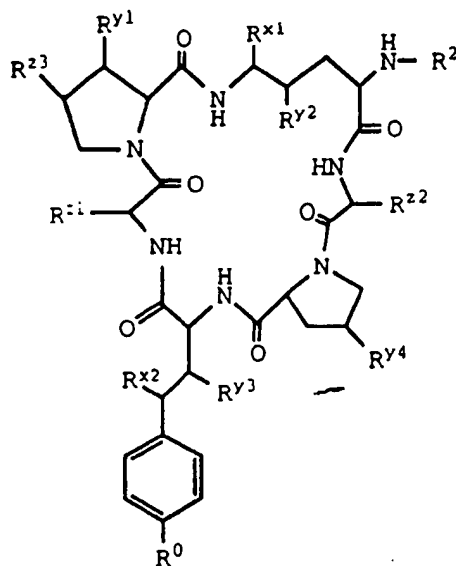
(74) Representative: **Tapping, Kenneth George et al**
Lilly Industries Limited
European Patent Operations
Erl Wood Manor
Windlesham Surrey GU20 6PH (GB)

(30) Priority: **25.07.1995 US 506790**

(71) Applicant: **ELI LILLY AND COMPANY**
Indianapolis, Indiana 46285 (US)

(54) Cyclic peptide anti-fungal agents

(57) Provided are pharmaceutical formulations, and methods of inhibiting fungal and parasitic activity using a compound of formula I:

**I**

wherein:

- R¹ is hydrogen, -CH₂OH, -CHOHCH₃ or -CHOHCH₂C(O)NH₂;
- R² is hydrogen, -CH₂OH or -CHOHCH₃;
- R³ is hydrogen or methyl;
- R^{x1} is hydrogen, hydroxy or O-R^{x1'};
- R^{x1'} is C₁-C₆ alkyl, benzyl, -(CH₂)₂Si(CH₃)₃, -CH₂CH=CH₂, -CH₂CHOHCH₂OH, -(CH₂)_aCOOH, -(CH₂)_bNR^{w1}R^{w2}, -(CH₂)_cOR^{w3}R^{w4} or -[(CH₂)₂O]_d-(C₁-C₆)alkyl;
- a, b and c are independently 1, 2, 3, 4, 5 or 6;
- R^{w1} and R^{w2} are independently hydrogen, C₁-C₆ alkyl, or R^{w1} and R^{w2} combine to form -CH₂(CH₂)₆CH₂-;

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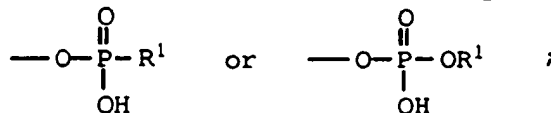
R^{w3} and R^{w4} are independently hydroxy, or C₁-C₆ alkoxy;

d is 1 or 2;

e is 1, 2 or 3;

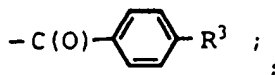
R^{x2}, R^{y1}, R^{y2}, R^{y3} and R^{y4} are independently hydroxy or hydrogen;

R⁰ is hydroxy, -OP(O)(OH)₂ or a group of the formulae:

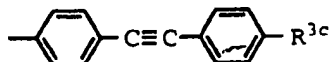
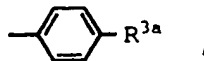


R¹ is C₁-C₆ alkyl, phenyl, p-halo-phenyl, p-nitrophenyl, benzyl, p-halo-benzyl or p-nitro-benzyl;

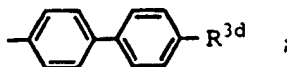
R² is



R³ is



or



R^{3a}, R^{3b}, R^{3c} and R^{3d} are independently hydrogen, C₁-C₁₂ alkyl, C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkylthio, halo, -O-(CH₂)_m-[O-(CH₂)_n]_p-O-(C₁-C₁₂ alkyl) or -O-(CH₂)_q-X-R⁴;

m is 2, 3 or 4;

n is 2, 3 or 4;

p is 0 or 1;

q is 2, 3 or 4;

X is pyrrolidino, piperidino or piperazino; and

R⁴ is hydrogen, C₁-C₁₂ alkyl, C₃-C₁₂ cycloalkyl, benzyl or C₃-C₁₂ cycloalkylmethyl;

with the proviso that at least one of R^{z1} and R^{z2} must be hydrogen;

or a pharmaceutically acceptable salt thereof.

Description

This invention relates to semi-synthetic cyclic peptide compounds which are useful as antifungal agents and which have improved stability and water solubility. In particular, it relates to derivatives of the echinocandin class of cyclic peptides; to methods for treating fungal infections, and to formulations useful in the methods.

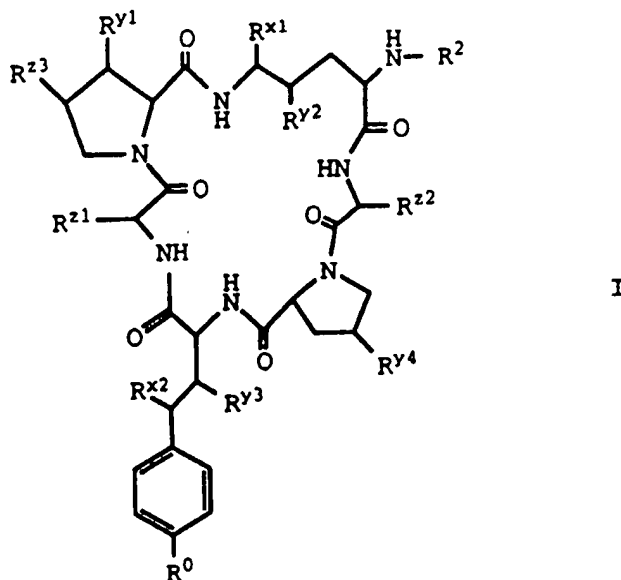
The compounds provided by this invention are semi-synthetic compounds derived from cyclic peptides which are produced by culturing various microorganisms. A number of cyclic peptides are known in the art including echinocandin B (A30912A), aculeacin, mulundocandin, sporiofungin and 531794/F1.

In general, these cyclic peptides may be structurally characterized as a cyclic hexapeptide core (or nucleus) with an acylated amino group on one of the core amino acids. The amino group is typically acylated with a fatty acid group forming a side chain off the nucleus. For example, echinocandin B has a linoleoyl side chain while aculeacin has a palmitoyl side chain.

The fatty acid side chains may be removed from the cyclic peptide core to provide an amino nucleus (for example, a compound of formula I, below, where R^2 is hydrogen). The amino group may then be re-acylated to provide semi-synthetic compounds such as those claimed in the present application.

The echinocandin B nucleus has been re-acylated with certain non-naturally occurring side chain moieties to provide a number of antifungal agents (see, Debono, U.S. Pat. Ser. No. 4,293,489). Among such antifungal agents is cilofungin which is represented by a compound of formula I where R^{z1} and R^{z2} are each $-\text{CHOHCH}_3$, R^{z3} is methyl; R^{x1} is hydrogen, R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 is hydroxy and R_2 is p-(octyloxy)benzoyl.

The present invention provides a compound of formula I:



wherein:

R^{z1} is hydrogen, $-\text{CH}_2\text{OH}$, $-\text{CHOHCH}_3$ or $-\text{CHOHCH}_2\text{C}(\text{O})\text{NH}_2$;

R^{z2} is hydrogen, $-\text{CH}_2\text{OH}$ or $-\text{CHOHCH}_3$;

R^{z3} is hydrogen or methyl;

R^{x1} is hydrogen, hydroxy or $\text{O}-R^{x1'}$;

$R^{x1'}$ is C_1 - C_6 alkyl, benzyl, $-(\text{CH}_2)_2\text{Si}(\text{CH}_3)_3$, $-\text{CH}_2\text{CH}=\text{CH}_2$, $-\text{CH}_2\text{CHOHCH}_2\text{OH}$, $-(\text{CH}_2)_a\text{COOH}$, $-(\text{CH}_2)_b\text{NR}^{w1}\text{R}^{w2}$,

$-(\text{CH}_2)_c\text{POR}^{w3}\text{R}^{w4}$ or $[(\text{CH}_2)_2\text{O}]_d-(\text{C}_1-\text{C}_6)\text{alkyl}$;

a , b and c are independently 1, 2, 3, 4, 5 or 6;

R^{w1} and R^{w2} are independently hydrogen, C_1 - C_6 alkyl, or R^{w1} and R^{w2} combine to form $-\text{CH}_2(\text{CH}_2)_e\text{CH}_2-$;

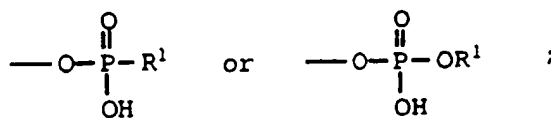
R^{w3} and R^{w4} are independently hydroxy, or C_1 - C_6 alkoxy;

d is 1 or 2;

e is 1, 2 or 3;

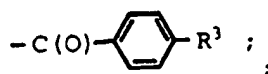
R^{x2} , R^{y1} , R^{y2} , R^{y3} and R^{y4} are independently hydroxy or hydrogen;

R⁰ is hydroxy, -OP(O)(OH)₂ or a group of the formulae:

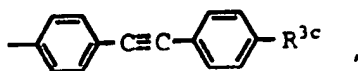
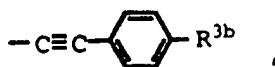
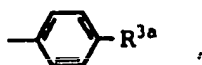


R¹ is C₁-C₆ alkyl, phenyl, p-halo-phenyl, p-nitrophenyl, benzyl, p-halo-benzyl or p-nitro-benzyl;

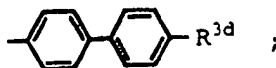
R² is



R³ is



or



R^{3a}, R^{3b}, R^{3c} and R^{3d} are independently hydrogen, C₁-C₁₂ alkyl, C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkylthio, halo, -O-(CH₂)_m-[O-(CH₂)_n]p-O-(C₁-C₁₂ alkyl) or -O-(CH₂)_q-X-R⁴;

m is 2, 3 or 4;

n is 2, 3 or 4;

p is 0 or 1;

q is 2, 3 or 4;

X is pyrrolidino, piperidino or piperazino; and

R⁴ is hydrogen, C₁-C₁₂ alkyl, C₃-C₁₂ cycloalkyl, benzyl or C₃-C₁₂ cycloalkylmethyl;

with the proviso that at least one of R²¹ and R²² must be hydrogen;
or a pharmaceutically acceptable salt thereof.

Also provided are pharmaceutical formulations, methods for inhibiting fungal activity and methods of treating fungal infections which employ the compounds of the invention.

As used herein, the term "C₁-C₁₂ alkyl" refers to a straight or branched alkyl chain having from one to twelve carbon atoms. Typical C₁-C₁₂ alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl, pentyl, 5-methylpentyl, hexyl, heptyl, 3,3-dimethylheptyl, octyl, 2-methyl-octyl, nonyl, decyl, undecyl, dodecyl and the like. The term "C₁-C₁₂ alkyl" includes within its definition the term "C₁-C₆ alkyl."

The term "halo" refers to chloro, fluoro, bromo or iodo.

The term " C_2 - C_{12} alkynyl" refers to a straight or branched alkynyl chain having from two to twelve carbon atoms. Typical C_2 - C_{12} alkynyl groups include ethynyl, 1-propyn-1-yl, 1-propyn-2-yl, 1-butyne-1-yl, 1-butyne-3-yl, 1-pentyne-3-yl, 4-pentyne-2-yl, 1-hexyn-3-yl, 3-hexyn-1-yl, 5-methyl-3-hexyn-1-yl, 5-octyn-1-yl, 7-octyn-1-yl, 4-decyn-1-yl, 6-decyn-1-yl and the like.

The term " C_1 - C_{12} alkylthio" refers to a straight or branched alkyl chain having from one to twelve carbon atoms attached to a sulfur atom. Typical C_1 - C_{12} alkylthio groups include methylthio, ethylthio, propylthio, isopropylthio, butylthio, 3-methyl-heptylthio, octylthio, 5,5-dimethylhexylthio and the like.

The term " C_1 - C_{12} alkoxy" refers to a straight or branched alkyl chain having from one to twelve carbon atoms attached to an oxygen atom. Typical C_1 - C_{12} alkoxy groups include methoxy, ethoxy, propoxy, butoxy, sec-butoxy, pentoxy, 5-methyl-hexoxy, heptoxy, octyloxy, decyloxy dodecyloxy and the like. The term " C_1 - C_{12} alkyl" includes within its definition the term " C_1 - C_6 alkoxy."

The term " C_3 - C_{12} cycloalkyl" refers to a saturated hydrocarbon ring structure having from three to twelve carbon atoms. Typical C_3 - C_{12} cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl, cyclooctyl and the like. The term " C_3 - C_{12} cycloalkylmethyl" refers to a C_3 - C_{12} cycloalkyl attached to a methylene group.

The term "amino-protecting group" as used in the specification refers to substituents of the amino group commonly employed to block or protect the amino functionality while reacting other functional groups on the compound. Examples of such amino-protecting groups include formyl, trityl, phthalimido, trichloroacetyl, chloroacetyl, bromoacetyl, iodoacetyl groups, or urethane-type blocking groups such as benzyloxycarbonyl, 4-phenylbenzyloxycarbonyl, 2-methylbenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 4-fluorobenzyloxycarbonyl, 4-chlorobenzyloxycarbonyl, 3-chlorobenzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, 4-bromobenzyloxycarbonyl, 3-bromobenzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 4-cyanobenzyloxycarbonyl, t-butoxycarbonyl, 2-(4-xenyl)isopropoxycarbonyl, 1,1-diphenylethyl-1-yloxycarbonyl, 1,1-diphenylprop-1-yloxycarbonyl, 2-phenylprop-2-yloxycarbonyl, 2-(p-toluy)l-prop-2-yloxycarbonyl, cyclopentanyloxycarbonyl, 1-methylcyclopentanyloxycarbonyl, cyclohexanyloxycarbonyl, 1-methylcyclohexanyloxycarbonyl, 2-methylcyclohexanyloxycarbonyl, 2-(4-toluy)sulfonyl)ethoxycarbonyl, 2-(methylsulfonyl)ethoxycarbonyl, 2-(triphenylphosphino)-ethoxycarbonyl, fluorenylmethoxycarbonyl ("Fmoc"), 2-(trimethylsilyl)ethoxycarbonyl, allyloxycarbonyl, 1-(trimethylsilylmethyl)prop-1-enyloxycarbonyl, 5-benzisoxalylmethoxycarbonyl, 4-acetoxybenzyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-ethynyl-2-propoxycarbonyl, cyclopropylmethoxycarbonyl, 4-(decyloxy)benzyloxycarbonyl, isobornyloxycarbonyl, 1-piperidyloxycarbonyl and the like; benzoylmethylsulfonyl, 2-nitrophenylsulfonyl, diphenylphosphine oxide and like amino-protecting groups. The species of amino-protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) on other positions of the intermediate molecule and can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other amino-protecting group(s). Preferred amino-protecting groups are t-butoxycarbonyl (t-Boc), allyloxycarbonyl and benzyloxycarbonyl (CbZ). Further examples of groups referred to by the above terms are described by J. W. Barton, "Protective Groups in Organic Chemistry", J. G. W. McOmie, Ed., Plenum Press, New York, N.Y., 1973, Chapter 2, and T. W. Greene, "Protective Groups in Organic Synthesis", John Wiley and sons, New York, N.Y., 1981, Chapter 7.

The term "hydroxy protecting group" refers to a substituent of an hydroxy group that is commonly employed to block or protect the hydroxy functionality while reactions are carried out on other functional groups on the compound. Examples of such hydroxy protecting groups include tetrahydropyranyl, 2-methoxyprop-2-yl, 1-ethoxyethyl-1-yl, methoxymethyl, β -methoxyethoxymethyl, methylthiomethyl, t-butyl, t-amyl, trityl, 4-methoxytrityl, 4,4'-dimethoxytrityl, 4,4', 4"-trimethoxytrityl, benzyl, allyl, trimethylsilyl, trimethylsilylethyl, (t-butyl)dimethylsilyl, and 2,2,2-trichloroethoxycarbonyl and the like. The species of hydroxy protecting group is not critical so long as the derivatized hydroxy group is stable to the conditions of the subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the molecule. A preferred hydroxy protecting group is trimethylsilylethyl. Further examples of hydroxy protecting groups are described in T.W. Greene, "Protective Groups in Organic Synthesis," John Wiley and Sons, New York, N.Y., (2nd ed., 1991) chapters 2 and 3. The term "protected hydroxy" refers to a hydroxy group bonded to one of the above hydroxy protecting groups.

The term "inhibiting", i.e. a method of inhibiting fungal activity, includes stopping, retarding or prophylactically hindering or preventing the growth or any attending characteristics and results from the existence of a fungus.

The term "contacting", i.e. contacting a compound of the invention with a fungus, includes a union or junction, or apparent touching or mutual tangency of a compound of the invention with a fungus. However, the term does not imply any further limitations to the process, such as by mechanism of inhibition, and the methods are defined to encompass the spirit of the invention, which is to inhibit fungal activity by the action of the compounds and their inherent antifungal properties, or in other words, the compounds, used in the claimed methods are the causative agent for such inhibition.

The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds of the above formula which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a mineral or organic acid or an inorganic base.

Such salts are known as acid addition and base addition salts.

Acids commonly employed to form acid addition salts are mineral acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid and the like, and organic acids such as p-toluenesulfonic, methanesulfonic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propionate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, γ -hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulfonic acid.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred.

It should be recognized that the particular counterion forming a part of any salt of this invention is not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

Preferred compounds of this invention are those compounds of formula I where:

R^{z1} is hydrogen, $-CH_2OH$ or $-CHOHCH_3$;

or a pharmaceutically acceptable salt thereof.

Of these compounds, more preferred are those compounds of formula I where:

R^{z1} and R^{z2} are independently hydrogen or $-CHOHCH_3$;

R^{z3} is methyl;

R^{y1} , R^{y2} , R^{y3} and R^{y4} are each hydroxy;

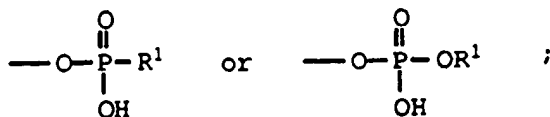
R^{x1} is hydrogen, hydroxy or $-OR^{x1'}$;

$R^{x1'}$ is methyl, benzyl, $-CH_2CHOHCH_2OH$, $-CH_2COOH$, $-(CH_2)_2NR^{w1}R^{w2}$ or $-(CH_2)_2POR^{w3}R^{w4}$;

R^{w1} and R^{w2} are independently hydrogen or methyl;

R^{w3} and R^{w4} are independently hydrogen or methoxy;

R^0 is hydroxy or a group of the formulae:



R^1 is methyl;

or a pharmaceutically acceptable salt thereof.

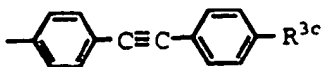
Of these preferred compounds, further preferred are those compounds of formula I where:

R^{z1} and R^{z2} are both hydrogen;

R^{x1} is hydrogen;

R^{x2} is hydrogen;

R^3 is



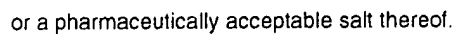
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or a pharmaceutically acceptable salt thereof.

 \mathbb{R}^3 is

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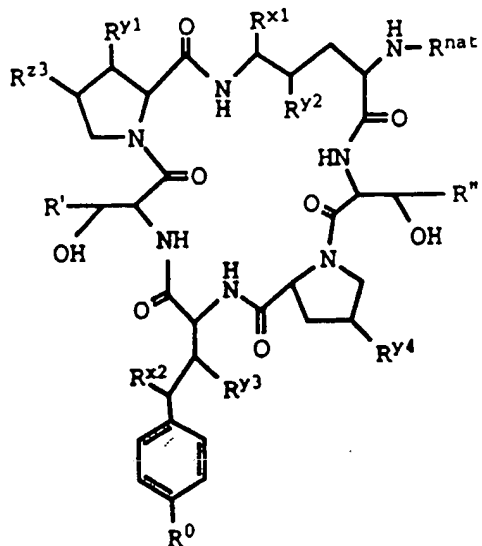
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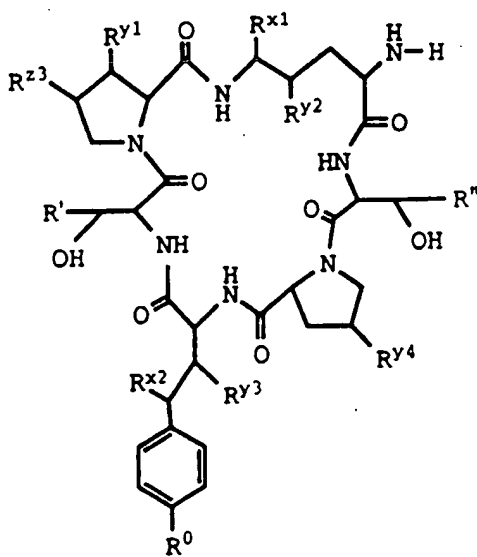
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Reaction Scheme I

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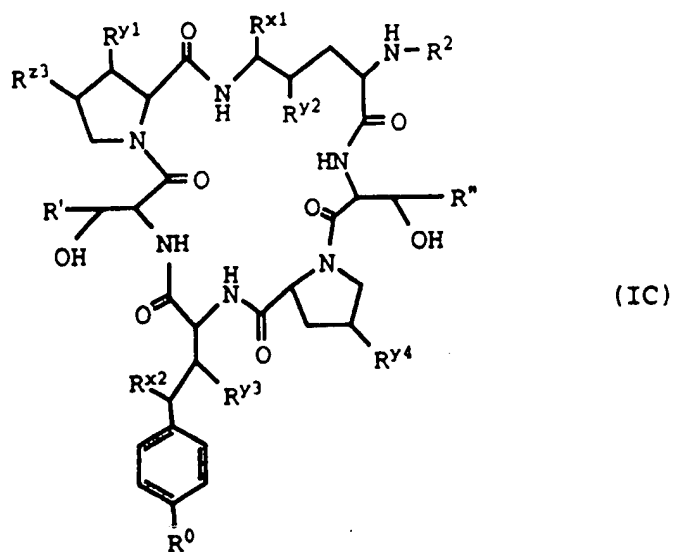
A. deacylate



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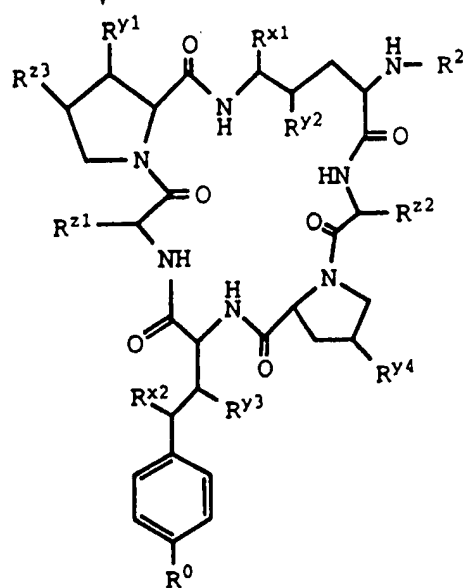
B. re-acylate

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C. Retro-aldol reaction



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wherein:

R^{nat} is a naturally occurring cyclic peptide sidechain;

R^1 is hydrogen, methyl or $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$;

R^* is hydrogen or methyl; and

R^{z1} , R^{z2} , R^{z3} , R^{x1} , $R^{x1'}$, R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} , R^0 and R^2 are as defined above.

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Reaction scheme I, above, is accomplished by carrying out reactions A-C, above. Once a reaction is complete, the intermediate compound may be isolated by procedures well-known in the art, for example, the compound may be crystallized or precipitated and then collected by filtration, or the reaction solvent may be removed by extraction, evap-

oration or decantation. The intermediate compound may be further purified, if desired, by common techniques such as crystallization or precipitation or chromatography over solid supports such as silica gel, alumina and the like, before carrying out the next step of the reaction scheme.

In reaction IA, a naturally occurring cyclic peptide of the formula IA is deacylated using procedures known in the art to provide an amino nucleus of formula IB. This reaction is typically carried out using enzymatic deacylation by exposing the naturally occurring cyclic peptide to a deacylase enzyme. The deacylase enzyme may be obtained from the microorganism *Actinoplanes utahensis* and used substantially as described in U.S. Patent Nos. 4,293,482 and 4,304,716, herein incorporated by reference. The deacylase enzyme may also be obtained from the *Pseudomonas* species. Deacylation may be accomplished using whole cells of *Actinoplanes utahensis* or *Pseudomonas* or the crude or purified enzyme thereof or using an immobilized form of the enzyme. See European Patent Application No. 0 460 882 (December 11, 1991). Examples of naturally occurring cyclic peptides which may be used as starting materials include aculeacin (palmitoyl side chain), tetrahydroechinocandin B (stearoyl side chain), mulundocandin (branched C₁₅ side chain), L-671,329 (C₁₆ branched side chain), S 31794/F1 (tetradecanoyl side chain), sporiofungin (C₁₅ branched side chain), FR901379 (palmitoyl side chain) and the like. A preferred naturally occurring cyclic peptide is echinocandin B (a compound of formula IA where R¹, R² and R²³ are each methyl, R^{x1}, R^{x2}, R^{y1}, R^{y2}, R^{y3}, R^{y4} and R⁰ are each hydroxy and R² is linoleoyl).

In Reaction IB, the amino nucleus of formula IB is re-acylated using procedures known in the art to provide a compound of formula IC where R² is an acyl group as defined hereinabove.

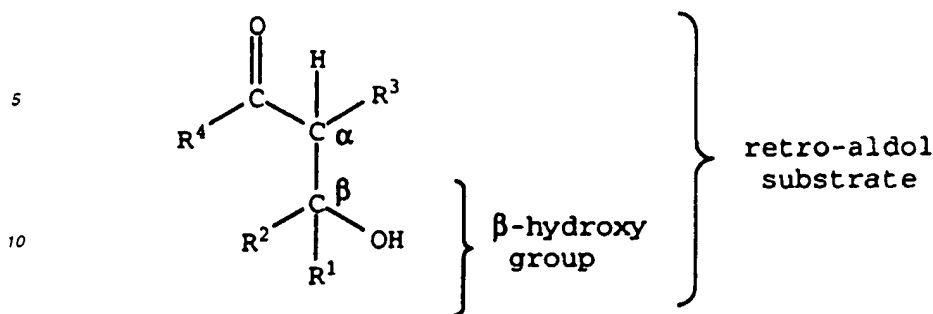
For example, the amino nucleus may be acylated by reaction with an appropriately substituted acyl halide, preferably in the presence of an acid scavenger such as a tertiary amine, such as triethylamine. The reaction is typically carried out at a temperature of from about -20°C to about 25°C. Typical solvents for this reaction include polar aprotic solvents such as dioxane or dimethylformamide. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction.

The amino nucleus may also be acylated by reaction with an appropriately substituted carboxylic acid, in the presence of a coupling agent. Typical coupling agents include dicyclohexylcarbodiimide (DCC), N,N'-carbonyldiimidazole, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and the like.

In addition, the amino nucleus may be acylated with an activated ester of a carboxylic acid such as an ester of a carboxylic acid of the formula R²-COOH and p-nitrophenyl, 2,4,5-trichlorophenyl, hydroxybenzotriazole hydrate (HOBT-H₂O), pentafluorophenol, N-hydroxysuccinimide and the like. Preferred acylating moieties are the active esters of the carboxylic acid R²-COOH such as 2,4,5-trichlorophenyl ester and benzotriazole ester. The reaction is typically carried out for one to sixty five hours at a temperature from about 0°C to about 30°C in an aprotic solvent. The reaction is generally complete after about twenty four to forty eight hours when carried out at a temperature of from about 15°C to about 30°C. Typical solvents for this reaction are tetrahydrofuran and dimethylformamide or a mixture of such solvents. The amino nucleus is generally employed in equimolar proportions relative to the activated ester or with a slight excess of the amino nucleus.

In Reaction IC, one or both of the β-hydroxy groups are selectively removed from a compound of formula IC to provide a compound of formula I. Specifically, the compound of formula IC is heated in an aprotic solvent to between about 50°C and 100°C in the presence of at least about five equivalents (preferably at least ten equivalents) of a retro-aldol-promoting reagent selected from the group consisting of trimethylamine-N-oxide, triethylamine-N-oxide, trimethylamine-N-oxide-hydrate and trimethylamine-N-oxide-hydrate. The reaction is generally conducted in a sealed tube or in a round bottom flask supplied with a water condenser for approximately three to seventy two hours. The reaction is preferably conducted at a temperature of about 70°C to about 100°C, preferably at about 70°C. Typical aprotic solvents include dimethylformamide, tetrahydrofuran, acetonitrile, dimethylsulfoxide and the like. A preferred solvent is acetonitrile. Preferred retro-aldol-promoting reagents are trimethylamine-N-oxide and trimethylamine-N-oxide-hydrate. The most preferred retro-aldol-promoting reagent is trimethylamine-N-oxide-hydrate. This reaction is preferably conducted after protecting the aminal hydroxy (R^{x1}) of the compound of formula IC. The hydroxy protecting group may be removed after removal of the β-hydroxy group(s), using procedures known in the art.

The term "aldol" indicates a molecule that is both an alcohol and an aldehyde or ketone wherein the hydroxyl and carbonyl functional groups are on adjacent carbon atoms as shown below. Thus, as used herein, the term "β-hydroxy group" refers to the alcohol moiety including both the β-carbon and the hydroxyl functional group that is resident on the β-carbon according to the following general structure:



15 where:

R¹ and R² are independently hydrogen or methyl;

R³ and R⁴ taken together with the atoms to which they are attached form a cyclic peptide.

20 The compounds where R^{x1} is -O-R^{x1'} may be prepared by the corresponding compound where R^{x1} is hydroxy with an appropriately substituted alcohol in the presence of an acid to provide a compound of formula I wherein R^{x1} is -O-R^{x1'} where R^{x1'} is C₁-C₆ alkyl, benzyl, -(CH₂)₂Si(CH₃)₃, -CH₂CH=CH₂, -(CH₂)_aCOOH, -(CH₂)_bNR^{w1}R^{w2}, -(CH₂)_cPOR^{w3}R^{w4} or -[(CH₂)₂O]_d-(C₁-C₆)alkyl. The compounds where R^{x1} is -O-R^{x1'} are preferably prepared after removal of the β-hydroxy group(s). The reaction is typically carried out in a polar aprotic solvent such as dioxane or dimethylsulfoxide at a temperature of from about 0°C to about 35°C, preferably at about room temperature. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction. Preferred acids include p-toluenesulfonic acid, hydrochloric acid and camphorsulfonic acid.

30 The compounds where R^{x1'} is -(CH₂)_bNR^{w1}R^{w2} where R^{w1} and R^{w2} are hydrogen may be prepared via a protected compound wherein R^{x1} is -(CH₂)_bNHR^a where R^a is an amino protecting group. The resultant protected compound may be deprotected according to procedures known in the art.

35 The compounds where R^{x1'} is -CH₂CHOHCH₂OH may be prepared by hydroxylating a compound of formula I where R^{x1} is -CH₂CH=CH₂ with osmium tetroxide in the presence of a catalyst at a temperature in the range of from about 0°C to about 40°C for about one to twenty four hours in a organic/aqueous solvent mixture, for example dioxane/water. Suitable catalysts include N-methylmorpholine N-oxide (NMO) and the like. Typical solvents suitable for use in this reaction include dimethylformamide, tetrahydrofuran, ethyl acetate, dioxane or a mixture of these solvents. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction. The reaction is preferably conducted at a temperature in the range of from about 20°C to about 30°C for about eighteen to twenty four hours.

40 The compounds where R⁰ is hydroxy may be phosphorylated by reaction with an appropriately substituted alkyl, phenyl or benzyl phosphate to provide a compound where R⁰ is -O-P(O)OH-OR¹ where R¹ is C₁-C₆ alkyl, phenyl or benzyl, or by reaction with an appropriately substituted alkyl, phenyl or benzyl phosphonic acid to provide a compound where R⁰ is -O-P(O)OH-R¹ where R¹ is C₁-C₆ alkyl, phenyl or benzyl, to provide a compound of formula I where R⁰ is a group of the formula -OP(O)OH-OR¹ or -OP(O)OH-R¹. This reaction is preferably conducted after removal of the β-hydroxy group(s). The phosphonic acid is typically used in an activated form, for example as a phosphonic halide, preferably a phosphonic chloride. The reaction is carried out in the presence of a base such as lithium trimethylsilanolate (LiOTMS), lithium bis(trimethylsilyl)amide (LHMDS), pyridine and the like. The reaction is typically carried out for up to one hour at a temperature from about -30°C to about 0°C in an aprotic solvent such as tetrahydrofuran and dimethylformamide. The reaction is generally complete in about fifteen minutes when carried out under these conditions. The phosphate or phosphonate reactant is generally employed in equimolar proportions to about a one mole excess relative to the amino nucleus in the presence of an equimolar or slight excess of the base. Phosphorylation of an amino nucleus with unprotected aminal hydroxy groups is typically carried out at lower temperatures, for example from about -30°C to about -15°C.

55 Alternatively, the compounds where R^{x1} is hydroxy may be phosphorylated after first protecting the hydroxy moiety with an hydroxy protecting group using procedures known in the art. For example, the reaction is typically carried out by combining the compound with a suitable hydroxy protecting group in the presence of a catalyst at a temperature in the range of from about 0°C to about 40°C for about one to five hours in a mutual inert solvent. The hydroxy protecting group is generally employed in an amount ranging from about equimolar proportions to about a 100 molar excess

relative to the compound, preferably in a large molar excess. Suitable catalysts include strong acids such as p-toluenesulfonic acid, camphorsulfonic acid (CSA), hydrochloric acid, sulfuric acid, trifluoroacetic acid and the like. Typical solvents suitable for use in this reaction include any organic solvent such as dioxane. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction. The reaction is preferably conducted at a temperature in the range of from about 20°C to about 30°C for about two to four hours. The protected compound is then phosphorylated as described above. The hydroxy protecting group is then removed according to procedures known in the art to provide the desired phosphorylated compound. For example, the protecting groups can be removed by reaction with a Lewis acid in a mutual inert organic solvent such as methylene chloride. Examples of Lewis acids include trimethylsilylbromide, boron trifluoride etherate and the like. The reaction is typically carried out at a temperature of from about 0°C to about 40°C, preferably at a temperature of from about 20°C to about 30°C. A preferred Lewis acid is boron trifluoride etherate.

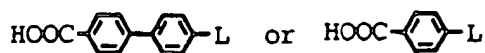
The dideoxy compounds are prepared by removing the benzylic and aminor hydroxy groups (R^{x2} and R^{x1} , respectively). The hydroxy groups may be removed by subjecting a non-dideoxy compound to a strong acid and a reducing agent at a temperature of between -5°C and 70°C, in a suitable solvent. Typical strong acids include trichloroacetic acid, trifluoroacetic acid or borontrifluoride etherate. A preferred strong acid is trifluoroacetic acid. Typical reducing agents include sodium cyanoborohydride or triethylsilane. A preferred reducing agent is triethylsilane. Suitable solvents include methylene chloride, chloroform or acetic acid, preferably methylene chloride. The strong acid should be present in an amount of from 2 to 80 mol per mol of substrate, and the reducing agent should be present in an amount of 2 to 80 mol per mol of substrate. This process affords selective removal of the aminor and benzylic hydroxy groups.

The cyclic peptides used to make the compounds of the present invention may be prepared by fermentation of known microorganisms. For example, the cyclic peptide of formula IB where R^1 , R^* and R^{z3} are methyl, and R^{x1} , R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 are each hydroxy (cyclic nucleus corresponding to A-30912A) may be prepared using the procedure detailed in Abbott et al., U.S. Pat. Ser. No. 4,293,482, which is herein incorporated by reference. The cyclic peptide of formula IB where R^1 , R^* and R^{z3} are methyl, R^{x1} is hydroxy, R^{x2} is hydrogen, and R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 are each hydroxy (cyclic nucleus corresponding to A-30912B) may be prepared using the procedure detailed in Abbott et al., U.S. Pat. Ser. No. 4,299,763, which is herein incorporated by reference. Aculeacin may be prepared using the procedure detailed in Mizuno et al., U.S. Pat. Ser. No. 3,978,210 which is herein incorporated by reference. The cyclic peptide of formula IB where R^1 is $-CH_2C(O)NH_2$, R^* is methyl, R^{z3} is hydrogen, and R^{x1} , R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 are each hydroxy may be prepared by deacylating the cyclic peptide prepared using the procedure detailed in Chen et al., U.S. Pat. Ser. No. 5,198,421, which is herein incorporated by reference.

The R^2 -COOH precursor acids, used to acylate the amino nucleus of formula IB, may be obtained commercially or prepared according to procedures known in the art. For example, an appropriately substituted phenyl boronic acid or biphenyl boronic acid reactant may be reacted with a p-halobenzoic acid reactant in the presence of a catalyst such as tetrakis(triphenylphosphine)palladium and an inorganic base such as potassium carbonate in a mutual inert organic solvent such as toluene at a temperature of from about 20°C to the reflux temperature of the reaction mixture to provide the corresponding biphenyl carboxylic acids and terphenyl carboxylic acids used to prepare the compounds of formula I. The reaction is typically carried out with equimolar proportions of the boronic acid reactant and the p-benzoic acid reactant, or a slight molar excess of the p-benzoic acid reactant relative to the boronic acid reactant, and a 1-2 molar excess of the inorganic base. The reaction is generally complete after about four to about ten hours when carried out at reflux temperature in toluene.

The boronic acid reactant may be prepared by reacting an appropriately substituted halophenyl or halobiphenyl reactant with two equivalents of triisopropyl borate in the presence of an alkyl lithium, for example sec-butyl lithium, in a mutual inert solvent such as tetrahydrofuran. The alkyl lithium is typically employed in a slight molar excess relative to the halophenyl or halobiphenyl reactant. The alkyl lithium is typically combined with the solvent by dropwise addition at reduced temperatures (<-70°C) and allowed to stir for approximately thirty minutes before the addition of the triisopropyl borate. The reaction is typically carried out initially at a temperature of from about -100°C to about -50°C, preferably from about -75°C to about -85°C for thirty minutes to two hours and then warmed to room temperature and reacted for an additional one to three hours. The reaction is generally complete in from several minutes to about four hours. When the reaction is substantially complete, the boronic acid moiety is formed by the addition of an acid. A preferred acid is a 1N hydrochloric acid solution.

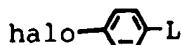
The carboxylic acids, R^2 -COOH, having an acetylene moiety may be prepared by reacting an appropriately substituted acetylene reactant with an appropriately substituted phenyl or biphenyl reactant of the formula



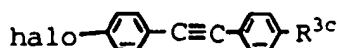
where L is a suitable leaving group such as bromo, iodo, methanesulfonate, toluenesulfonate, trifluoromethanesul-

fonate and the like, in the presence of a catalyst and preferably in the presence of an acid scavenger in a mutual inert solvent such as acetonitrile. Examples of acid scavengers include triethylamine and pyridine, preferably triethylamine. A preferred catalyst is formed *in situ* from palladium (II) chloride, triphenylphosphine and copper (I) iodide. The reaction is typically carried out for thirty minutes to twenty one hours at a temperature from about room temperature to the reflux temperature of reaction mixture. The reaction is generally complete after about two to about six hours when carried out at reflux temperature.

Alternatively, a suitably substituted phenyl reactant of the formula



may be reacted with an appropriately substituted acetylene reactant as described above to provide, for example, a compound of the formula

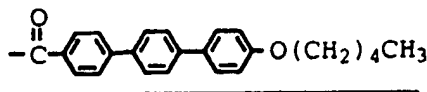


which can be coupled with a phenyl boronic acid reactant as described above.

The following Preparations and Examples further describe how to synthesize the compounds of the present invention. The terms melting point, proton nuclear magnetic resonance spectra, mass spectra, infrared spectra, ultraviolet spectra, elemental analysis, high performance liquid chromatography, and thin layer chromatography are abbreviated "m.p.", "NMR", "MS", "IR", "UV", "Analysis", "HPLC", and "TLC", respectively. In addition, the absorption maxima listed for the IR spectra are only those of interest and not all of the maxima observed.

Example 1

A. Preparation of the compound of formula 1C where R¹, R² and R²³ are each methyl, R^{x1} and R^{x2} are each hydroxy, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is

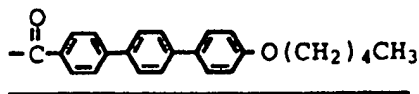


The N-acyl cyclic peptide derivatives listed in Table 3, below were prepared by dissolving 348.1 g (60.2 mmol) of the A30912A nucleus (compound of formula 1B where R¹, R² and R²³ are each methyl, R^{x1} and R^{x2} are each hydroxy, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, and R⁰ is hydroxy) and 26.0 g (48.2 mmol) of the 2,4,5-trichlorophenol ester of [[[4-(4-pentyloxy)-1,1':4',1''-terphenyl]-4-carboxylic acid, in 8.5 l of dimethylformamide. The resultant reaction mixture was stirred for approximately 48 hours at room temperature and then the solvent was removed *in vacuo* to provide a residue. This residue was slurried in ether, collected by filtration, washed with methylene chloride and then dissolved in methanol or a 1:1 (v/v) acetonitrile/water mixture. The resultant solution is subjected to reverse phase HPLC (C18; eluent of 20-40% aqueous acetonitrile containing 0.5% monobasic ammonium phosphate (w/v); 20 ml/min.; 230 nm). After removing the unreacted A30912A nucleus, the desired product was eluted from the column using an eluent of aqueous acetonitrile. The fractions containing the desired product are combined and then reduced to dryness *in vacuo* to provide the desired acylated nucleus.

Yield: 18 g.

MS(FAB) : 1140.5103 (M⁺¹).

B. Preparation of the compound of formula IC where R¹, R^a and R^{z3} are each methyl, R^{x1} and R^{x2} are each hydrogen, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is



To a mixture of 5 g (4.4 mmol) of the compound prepared in Example 1A and 17 ml of trifluoroacetic acid in 250 ml of methylene chloride, was added 35 ml of triethylsilane. When the reaction was substantially complete, as indicated by HPLC (C18, eluent of 55% acetonitrile; 2 ml/min; 280 nm; R_T (starting material) = 4.19 min.; R_T (product) = 6.40 min.), the reaction mixture was concentrated *in vacuo* to provide a solid. This solid was slurried in 100 ml of 50% aqueous acetone and then dissolved by adjusting the pH of the mixture to approximately pH 7. The resultant solution was poured into a large volume of water (approximately 1 liter) resulting in the precipitation of a white solid. This solid was isolated by filtration through a sintered glass funnel, washed with diethyl ether and then dried *in vacuo* at 55°C to provide 3.718 g of the titled compound. The funnel was washed with methanol to collect the remaining solid, which was dried *in vacuo* to provide an additional 0.154 g of the titled compound.

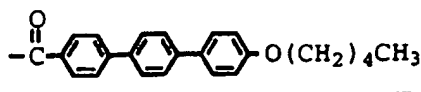
Yield: 3.872 g (79%).

MS (FAB): m/e 1108.7 (M)

HPLC: (eluent of 55% acetonitrile; 2 ml/min.; 280 nm):

R_T = 6.43 min.

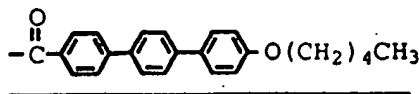
C. Preparation of the compound of formula I where R^{z1} and R^{z2} are each hydrogen, R^{z3} is methyl, R^{x1} and R^{x2} each hydrogen, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is



The compound prepared in Example 1B was dissolved in a 1:1 mixture of acetonitrile and dimethylformamide. To this solution, was added TNO-hydrate (1.6 g, 14.3 mmol) all at once. The reaction mixture was heated at 100°C for 48 hours after which the mixture was cooled to room temperature and concentrated to approximately one-half its original volume. The crude residue was dissolved with acetic acid and purified by reverse phase preparative HPLC to yield 150 mg final product (52%). TNO-hydrate converted the two threonine residues into glycine residues and left the homotyrosine untouched as confirmed by fast atom bombardment mass spectrometry, which confirmed the molecular weight of the product having the chemical formula C₅₄H₆₅N₇O₁₃: calculated - 1020.1; found - 1020.8. (M+H).

Example 2

Alternative Preparation of the compound of formula I where R^{z1} and R^{z2} are each hydrogen, R^{z3} is methyl, R^{x1} and R^{x2} are each hydrogen, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is



The compound prepared in Example 1B was dissolved in 20 ml of a 1:1 mixture of acetonitrile and dimethylformamide. To this solution, was added trimethylamine N-oxide dihydrate (2.51 g, 22.6 mmol) all at once. The reaction mixture was heated at 100°C for 24 hours in a sealed tube after which the mixture was cooled to room temperature and concentrated *in vacuo* to approximately one-half its original volume. The crude residue was isolated by filtration and then rinsed with cold dimethylformamide. The resultant filtrate was dried *in vacuo* to provide a residue. This residue was purified using reverse phase HPLC (3x40x10 radial column, eluent of 50% acetonitrile in water, 60 ml/min., 280 nm) to yield 330 mg final product (72%).

Fast atom bombardment mass spectrometry confirmed the molecular weight of the product having the chemical formula C₅₄H₆₅N₇O₁₃: calculated - 1020.1; found - 1020.4 (M+H).

The compounds of formula I exhibit antifungal activity. For example, the compounds of formula I inhibit the growth of various infectious fungi including *Candida* spp. such as *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, or *C. tropicalis*, *C. lusitanae*; *Torulopus* spp. such as *T. glabrata*; *Aspergillus* spp. such as *A. fumigatus*; *Histoplasma* spp. such as *H. capsulatum*; *Cryptococcus* spp. such as *C. neoformans*; *Blastomyces* spp. such as *B. dermatitidis*; *Fusarium* spp., *Trichophyton* spp., *Pseudallescheria boydii*, *Coccidioides immitis*, *Sporothrix schenckii* and the like.

Antifungal activity of a test compound is determined *in vitro* by obtaining the minimum inhibitory concentration (MIC) of the compound using a standard agar dilution test or a disc-diffusion test. The compound is then tested *in vivo* (in mice) to determine the effective dose of the test compound for controlling a systemic fungal infection.

Accordingly, the compound prepared in Example 1C was tested for antifungal activity against *C. albicans*. The minimal inhibitory concentration (MIC) of this compound against *C. albicans* was 0.312 (μ g/ml).

In addition, the effective dose of the compound prepared in Example 1C, for controlling a systemic fungal infection (*C. albicans*), was tested *in vivo* (mice). The ED₅₀ for this compound was >2.5.

The compounds of formula I are active *in vitro* and *in vivo* and are useful in combating either systemic fungal infections or fungal skin infections. Accordingly, the present invention provides a method of inhibiting fungal activity comprising contacting a compound of formula I, or a pharmaceutically acceptable salt thereof, with a fungus. A preferred method includes inhibiting *Candida albicans* or *Aspergillus fumigatus* activity. The present invention further provides a method of treating a fungal infection which comprises administering an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof, to a host in need of such treatment. A preferred method includes treating a *Candida albicans* or *Aspergillus fumigatus* infection.

With respect to antifungal activity, the term "effective amount," means an amount of a compound of the present invention which is capable of inhibiting fungal activity. The dose administered will vary depending on such factors as the nature and severity of the infection, the age and general health of the host and the tolerance of the host to the antifungal agent. The particular dose regimen likewise may vary according to such factors and may be given in a single daily dose or in multiple doses during the day. The regimen may last from about 2-3 days to about 2-3 weeks or longer. A typical daily dose (administered in single or divided doses) will contain a dosage level of from about 0.01 mg/kg to about 100 mg/kg of body weight of an active compound of this invention. Preferred daily doses generally will be from about 0.1 mg/kg to about 60 mg/kg and ideally from about 2.5 mg/kg to about 40 mg/kg.

The present invention also provides pharmaceutical formulations useful for administering the antifungal compounds of the invention. Accordingly, the present invention also provides a pharmaceutical formulation comprising one or more pharmaceutically acceptable carriers, diluents or excipients and a compound of claim 1. The active ingredient in such formulations comprises from 0.1% to 99.9% by weight of the formulation, more generally from about 10% to about 30% by weight. By "pharmaceutically acceptable" it is meant that the carrier, diluent or excipient is compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

A compound of formula I may be administered parenterally, for example using intramuscular, subcutaneous, or intra-peritoneal injection, nasal or oral means. In addition to these methods of administration, a compound of formula I may be applied topically for skin infections.

For parenteral administration the formulation comprises a compound of formula I and a physiologically acceptable diluent such as deionized water, physiological saline, 5% dextrose and other commonly used diluents. The formulation may contain a solubilizing agent such as a polyethylene glycol or polypropylene glycol or other known solubilizing agent. Such formulations may be made up in sterile vials containing the antifungal and excipient in a dry powder or lyophilized powder form. Prior to use, a physiologically acceptable diluent is added and the solution withdrawn via syringe for administration to the patient.

The present pharmaceutical formulations are prepared by known procedures using known and readily available ingredients. In making the compositions of the present invention, the active ingredient will generally be admixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders and the like.

For oral administration, the antifungal compound is filled into gelatin capsules or formed into tablets. Such tablets may also contain a binding agent, a dispersant or other suitable excipients suitable for preparing a proper size tablet for the dosage and particular antifungal compound of the formula I. For pediatric or geriatric use the antifungal compound may be formulated into a flavored liquid suspension, solution or emulsion. A preferred oral formulation is linoleic acid, cremophor RH-60 and water and preferably in the amount (by volume) of 8% linoleic acid, 5% cremophor RH-60, 87% sterile water and a compound of formula I in an amount of from about 2.5 to about 40 mg/ml.

For topical use the antifungal compound may be formulated with a dry powder for application to the skin surface or it may be formulated in a liquid formulation comprising a solubilizing aqueous liquid or non-aqueous liquid, e.g., an

alcohol or glycol.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way. The term "active ingredient" means a compound according to formula I or a pharmaceutically acceptable salt thereof.

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active ingredient	250
Starch, dried	200
Magnesium stearate	10
Total	<u>460 mg</u>

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	<u>5</u>
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

An aerosol solution is prepared containing the following components:

	Weight
Active ingredient	0.25
Methanol	25.75
Propellant 22 (Chlorodifluoromethane)	74.00
Total	<u>100.00</u>

The active compound is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

Tablets, each containing 60 mg of active ingredient, are made as follows:

EP 0 757 058 A1

Active ingredient	60 mg
Starch	45 mg
Microcrystalline cellulose	35 mg
Polyvinylpyrrolidone (as 10% solution in water)	4 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1 mg
Total	150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The aqueous solution containing polyvinyl-pyrrolidone is mixed with the resultant powder, and the mixture then is passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

Capsules, each containing 80 mg of active ingredient, are made as follows:

Active ingredient	80 mg
Starch	59 mg
Microcrystalline cellulose	59 mg
Magnesium stearate	2 mg
Total	200 mg

The active ingredient, cellulose, starch and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

Suppositories, each containing 225 mg of active ingredient, are made as follows:

Active ingredient	225 mg
Saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

Formulation 7

Suspensions, each containing 50 mg of active ingredient per 5 ml dose, are made as follows:

Active ingredient	50 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 ml
Benzoic acid solution	0.10 ml
Flavor	q.v.
Color	q.v.
Purified water to total	5 ml

The active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with a portion of the water and added, with stirring. Sufficient water is then added to produce the required volume.

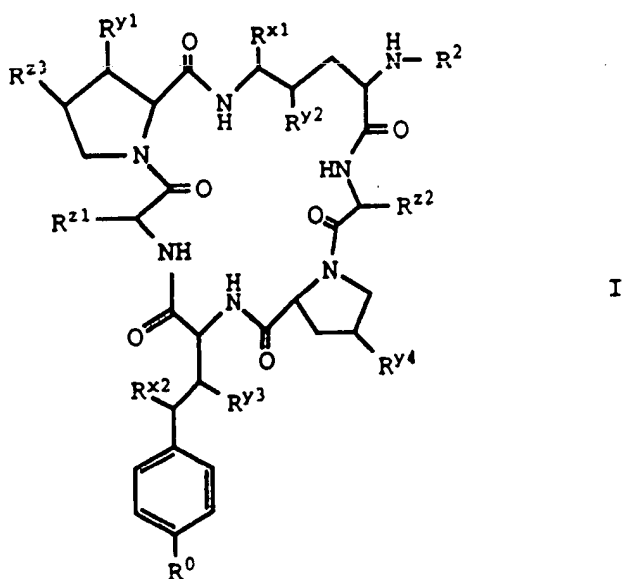
Formulation 8

An intravenous formulation may be prepared as follows: Active ingredient 100 mg
Isotonic saline 1,000 ml

The solution of the above ingredients generally is administered intravenously to a subject at a rate of 1 ml per minute.

Claims

1. A compound of formula I:



wherein:

R^{z1} is hydrogen, -CH₂OH, -CHOHCH₃ or -CHOHCH₂C(O)NH₂;

R^{z2} is hydrogen, -CH₂OH or -CHOHCH₃;

R^{z3} is hydrogen or methyl;

R^{x1} is hydrogen, hydroxy or O-R^{x1'};

R^{x1'} is C₁-C₆ alkyl, benzyl, -(CH₂)₂Si(CH₃)₃, -CH₂CH=CH₂, -CH₂CHOHCH₂OH, -(CH₂)_aCOOH, -(CH₂)_bNR^{w1}R^{w2}, -(CH₂)_cPOR^{w3}R^{w4} or -[(CH₂)₂O]_d-(C₁-C₆)alkyl;

a, b and c are independently 1, 2, 3, 4, 5 or 6;

R^{w1} and R^{w2} are independently hydrogen, C₁-C₆ alkyl, or R^{w1} and R^{w2} combine to form -CH₂(CH₂)_eCH₂-;

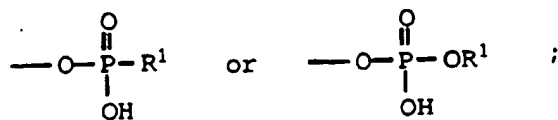
R^{w3} and R^{w4} are independently hydroxy, or C₁-C₆ alkoxy;

d is 1 or 2;

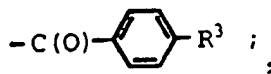
e is 1, 2 or 3;

R^{x2}, R^{y1}, R^{y2}, R^{y3} and R^{y4} are independently hydroxy or hydrogen;

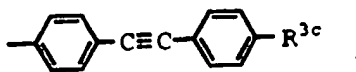
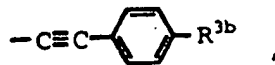
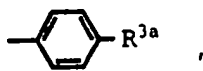
R⁰ is hydroxy, -OP(O)(OH)₂ or a group of the formulae:



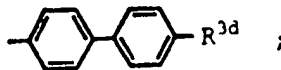
R^1 is C_1 - C_6 alkyl, phenyl, p-halo-phenyl, p-nitrophenyl, benzyl, p-halo-benzyl or p-nitro-benzyl;
 R^2 is



R^3 is



or



R^{3a} , R^{3b} , R^{3c} and R^{3d} are independently hydrogen, C_1 - C_{12} alkyl, C_2 - C_{12} alkynyl, C_1 - C_{12} alkoxy, C_1 - C_{12} alkylthio, halo, $\text{---O---(CH}_2\text{)}_m\text{---[O---(CH}_2\text{)}_n\text{]}_p\text{---O---(C}_1\text{---C}_{12}\text{ alkyl)}$ or $\text{---O---(CH}_2\text{)}_q\text{---X---R}^4$;

m is 2, 3 or 4;

n is 2, 3 or 4;

p is 0 or 1;

q is 2, 3 or 4;

x is pyrrolidino, piperidino or piperazino; and

R^4 is hydrogen, C_1 - C_{12} alkyl, C_3 - C_{12} cycloalkyl, benzyl or C_3 - C_{12} cycloalkylmethyl;

with the proviso that at least one of R^{z1} and R^{z2} must be hydrogen;
 or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 where:

R^{z1} is hydrogen, $\text{---CH}_2\text{OH}$ or ---CHOHCH_3 ;
 or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 2 where:

R^{z1} and R^{z2} are independently hydrogen or -CHOHCH₃;

R^{z3} is methyl;

R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy;

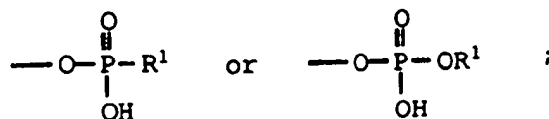
R^{x1} is hydrogen, hydroxy or -OR^{x1'};

R^{x1'} is methyl, benzyl, -CH₂CHOHCH₂OH, -CH₂COOH, -(CH₂)₂NR^{w1}R^{w2} or -(CH₂)₂POR^{w3}R^{w4};

R^{w1} and R^{w2} are independently hydrogen or methyl;

R^{w3} and R^{w4} are independently hydrogen or methoxy;

R⁰ is hydroxy or a group of the formulae:



R¹ is methyl;

or a pharmaceutically acceptable salt thereof.

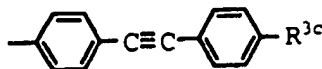
4. A compound according to claim 3 where:

R^{z1} and R^{z2} are both hydrogen;

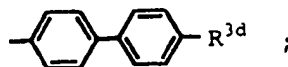
R^{x1} is hydrogen;

R^{x2} is hydrogen;

R³ is



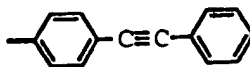
or



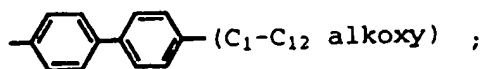
R^{3c} and R^{3d} are independently hydrogen, C₁-C₁₂ alkyl, C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy or -O-(CH₂)₂-O-(C₁-C₁₂ alkyl); or a pharmaceutically acceptable salt thereof.

5. A compound according to claim 4 where:

R³ is

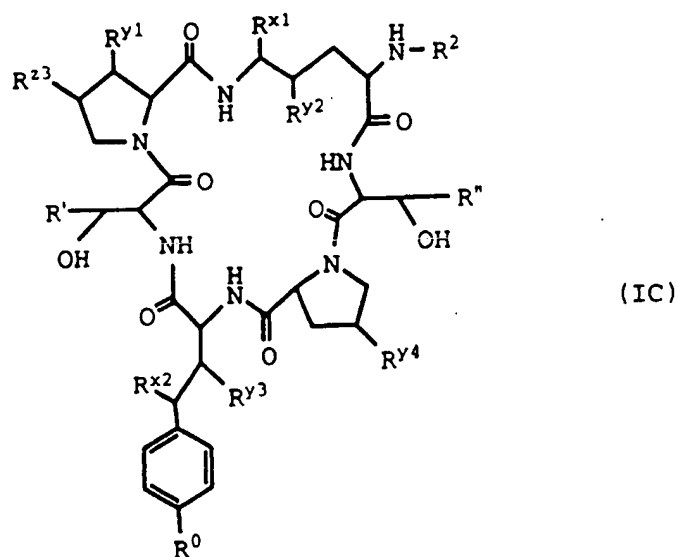


or



or a pharmaceutically acceptable salt thereof.

6. A pharmaceutical formulation comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 5, associated with one or more pharmaceutically acceptable carriers, diluents or excipients therefor.
7. A compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 5, for use as a pharmaceutical.
8. A process for preparing a compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 5, comprising heating a compound of formula IC:



wherein:

R', R'', R^{x1}, R^{x2}, R^{y1}, R^{y2}, R^{y3}, R^{y4} and R⁰ are as defined in claim 1;

in an aprotic solvent to between 50°C and 100°C in the presence of at least five equivalents of a retro-aldol-promoting reagent selected from the group consisting of trimethylamine-N-oxide, triethylamine-N-oxide, trimethylamine-N-oxide-hydrate and trimethylamine-N-oxide hydrate;

and then optionally converting the compound of formula I to a pharmaceutically acceptable salt thereof.



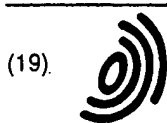
European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 96 30 5345

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	WO-A-96 15142 (LILLY CO ELI ; RODRIGUEZ MICHAEL JOHN (US)) 23 May 1996 * example 3 *	1-8	C07K7/56 A61K38/12
X	EP-A-0 561 639 (LILLY CO ELI) 22 September 1993 * claim 1; tables 1-29 *	1-3,6,7	
X	WO-A-95 08341 (MERCK & CO INC ; BALKOVEC JAMES M (US); BOUFFARD FRANCES A (US); DR) 30 March 1995 * claim 1; examples 1-3 *	1-3,6,7	
X	WO-A-94 25048 (MERCK & CO INC ; BALKOVEC JAMES M (US); BOUFFARD FRANCES AILEEN (US)) 10 November 1994 * claim 1; examples 1-6 *	1-3,6,7	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07K
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 29 October 1996	Examiner Deffner, C-A
CATEGORY OF CITED DOCUMENTS T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : technological background O : non-written disclosure P : intermediate document X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category & : member of the same patent family, corresponding document			

EPO FORM 1501 (01.91) (P04C01)



(19)

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(11)

EP 0 757 058 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
08.11.2000 Bulletin 2000/45

(51) Int Cl.7: **C07K 7/56, A61K 38/12**

(21) Application number: **96305345.9**

(22) Date of filing: **22.07.1996**

(54) Cyclic peptide anti-fungal agents

Zyklische Peptide mit antifungischer Wirkung

Dérivées cyclopeptidiques et antifongiques

(84) Designated Contracting States:
**AT BE CH DE DK ES FI FR GB GR IE IT LI LU NL
PT SE**
Designated Extension States:
AL LT LV SI

(30) Priority: **25.07.1995 US 506790**

(43) Date of publication of application:
05.02.1997 Bulletin 1997/06

(73) Proprietor: **ELI LILLY AND COMPANY**
Indianapolis, Indiana 46285 (US)

(72) Inventor: **Rodriguez, Michael John**
Indianapolis, Indiana 46260 (US)

(74) Representative: **Denholm, Anna Marie et al**
Eli Lilly and Company Limited,
Lilly Research Center,
Erl Wood Manor
Windsor, Surrey GU20 6PH (GB)

(56) References cited:
EP-A- 0 561 639 **WO-A-94/25048**
WO-A-95/08341 **WO-A-96/15142**

EP 0 757 058 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

[0001] This invention relates to semi-synthetic cyclic peptide compounds which are useful as antifungal agents and which have improved stability and water solubility. In particular, it relates to derivatives of the echinocandin class of cyclic peptides; to methods for treating fungal infections, and to formulations useful in the methods.

[0002] The compounds provided by this invention are semi-synthetic compounds derived from cyclic peptides which are produced by culturing various microorganisms. A number of cyclic peptides are known in the art including echinocandin B (A30912A), aculeacin, mulundocandin, sporiolungin and S31794/F1.

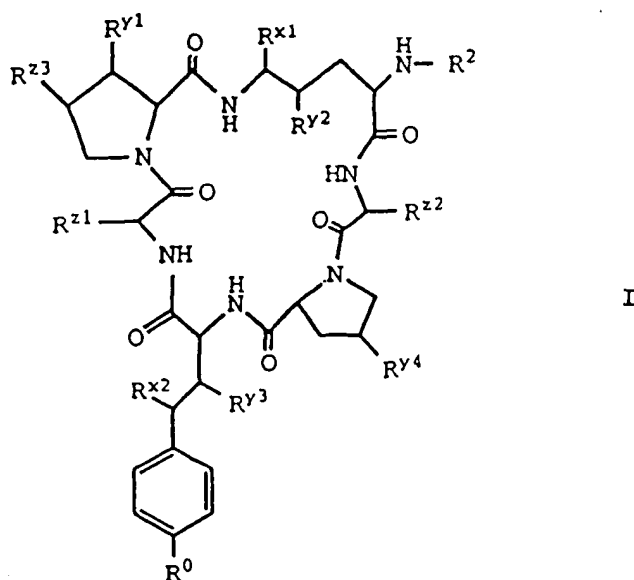
[0003] In general, these cyclic peptides may be structurally characterized as a cyclic hexapeptide core (or nucleus) with an acylated amino group on one of the core amino acids. The amino group is typically acylated with a fatty acid group forming a side chain off the nucleus. For example, echinocandin B has a linoleoyl side chain while aculeacin has a palmitoyl side chain.

[0004] The fatty acid side chains may be removed from the cyclic peptide core to provide an amino nucleus (for example, a compound of formula I, below, where R₂ is hydrogen). The amino group may then be re-acylated to provide semi-synthetic compounds such as those claimed in the present application.

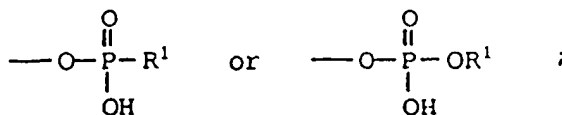
[0005] The echinocandin B nucleus has been re-acylated with certain non-naturally occurring side chain moieties to provide a number of antifungal agents (see, Debono, U.S. Pat. Ser. No. 4,293,489). Among such antifungal agents is cilofungin which is represented by a compound of formula I where R₂1 and R₂2 are each -CHOHCH₃, R₂3 is methyl; R₂1 is hydrogen, R₂2, R₂3, R₂4 and R₂0 is hydroxy and R₂ is p-(octyloxy)benzoyl.

[0006] WO 96/15142 discloses a process for removing β-hydroxy groups from β-hydroxy containing compounds such as complex cyclic peptides, linear peptides and non-peptides. WO 95/08341 discloses antifungal and anti-pneumocystis compounds with an Echinocandin B nucleus and with a polyphenylene side chain attached to the dihydroxyornithine residue at the N2 position and an aminoalkyl ether at the dihydroxyornithine residue at the C5 position. WO 94/25408 discloses certain amine compounds which have a cyclohexapeptidyl nucleus and which are found to have antibiotic activity. EP 0561639 discloses acyl derivatives of the Echinocandin class of cyclic peptide antifungal agents and process for preparation thereof.

[0007] The present invention provides a compound of formula I:

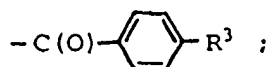


R⁰ is hydroxy, -OP(O)(OH)₂ or a group of the formulae:

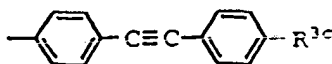


R¹ is C₁-C₆ alkyl, phenyl, p-halo-phenyl, p-nitrophenyl, benzyl, p-halo-benzyl or p-nitro-benzyl;

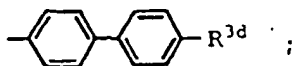
R² is



R³ is



or



R^{3c} and R^{3d} are independently hydrogen, C₁-C₁₂ alkyl, C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy or -O-(CH₂)₂-O-(C₁-C₁₂ alkyl);

or a pharmaceutically acceptable salt thereof.

[0008] Also provided are pharmaceutical formulations, methods for inhibiting fungal activity and methods of treating fungal infections which employ the compounds of the invention.

[0009] As used herein, the term "C₁-C₁₂ alkyl" refers to a straight or branched alkyl chain having from one to twelve carbon atoms. Typical C₁-C₁₂ alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl, pentyl, 5-methylpentyl, hexyl, heptyl, 3,3-dimethylheptyl, octyl, 2-methyl-octyl, nonyl, decyl, undecyl, dodecyl and the like. The term "C₁-C₁₂ alkyl" includes within its definition the term "C₁-C₆ alkyl."

[0010] The term "halo" refers to chloro, fluoro, bromo or iodo.

[0011] The term "C₂-C₁₂ alkynyl" refers to a straight or branched alkynyl chain having from two to twelve carbon atoms. Typical C₂-C₁₂ alkynyl groups include ethynyl, 1-propyn-1-yl, 1-propyn-2-yl, 1-butyne-1-yl, 1-butyne-3-yl, 1-pentyne-3-yl, 4-pentyne-2-yl, 1-hexyn-3-yl, 3-hexyn-1-yl, 5-methyl-3-hexyn-1-yl, 5-octyn-1-yl, 7-octyn-1-yl, 4-decyn-1-yl, 6-decyn-1-yl and the like.

[0012] The term "C₁-C₁₂ alkylthio" refers to a straight or branched alkyl chain having from one to twelve carbon atoms attached to a sulfur atom. Typical C₁-C₁₂ alkylthio groups include methylthio, ethylthio, propylthio, isopropylthio, butylthio, 3-methyl-heptylthio, octylthio, 5,5-dimethylhexylthio and the like.

[0013] The term "C₁-C₁₂ alkoxy" refers to a straight or branched alkyl chain having from one to twelve carbon atoms attached to an oxygen atom. Typical C₁-C₁₂ alkoxy groups include methoxy, ethoxy, propoxy, butoxy, sec-butoxy, pentoxy, 5-methyl-hexoxy, heptoxy, octyloxy, decyloxy dodecyloxy and the like. The term "C₁-C₁₂ alkyl" includes within its definition the term "C₁-C₆ alkoxy."

[0014] The term "C₃-C₁₂ cycloalkyl" refers to a saturated hydrocarbon ring structure having from three to twelve carbon atoms. Typical C₃-C₁₂ cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl, cyclooctyl and the like. The term "C₃-C₁₂ cycloalkylmethyl" refers to a C₃-C₁₂ cycloalkyl attached to a methylene group.

[0015] The term "amino-protecting group" as used in the specification refers to substituents of the amino group commonly employed to block or protect the amino functionality while reacting other functional groups on the compound. Examples of such amino-protecting groups include formyl, trityl, phthalimido, trichloroacetyl, chloroacetyl, bromoacetyl,

iodoacetyl groups, or urethane-type blocking groups such as benzyloxycarbonyl, 4-phenylbenzyloxycarbonyl, 2-methylbenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 4-fluorobenzyloxycarbonyl, 4-chlorobenzyloxycarbonyl, 3-chlorobenzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, 4-bromobenzyloxycarbonyl, 3-bromobenzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 4-cyanobenzyloxycarbonyl, *t*-butoxycarbonyl, 2-(4-xenyl)isopropoxycarbonyl, 1,1-diphenyleth-1-yloxycarbonyl, 1,1-diphenylprop-1-yloxycarbonyl, 2-phenylprop-2-yloxycarbonyl, 2-(*p*-toluyl)prop-2-yloxycarbonyl, cyclopentanyloxycarbonyl, 1-methylcyclopentanyloxycarbonyl, cyclohexanyloxycarbonyl, 1-methylcyclohexanyloxycarbonyl, 2-methylcyclohexanyloxycarbonyl, 2-(4-toluylsulfonyl)ethoxycarbonyl, 2-(methylsulfonyl)ethoxycarbonyl, 2-(triphenylphosphino)ethoxycarbonyl, fluorenylmethoxycarbonyl ("Fmoc"), 2-(trimethylsilyl)ethoxycarbonyl, allyloxycarbonyl, 1-(trimethylsilylmethyl)prop-1-enyloxycarbonyl, 5-benzisoxalylmethoxycarbonyl, 4-acetoxybenzyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, 2-ethynyl-2-propoxycarbonyl, cyclopropylmethoxycarbonyl, 4-(decyloxy)benzyloxycarbonyl, isobornyloxycarbonyl, 1-piperidyloxycarbonyl and the like; benzoylmethylsulfonyl, 2-nitrophenylsulfonyl, diphenylphosphine oxide and like amino-protecting groups. The species of amino-protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) on other positions of the intermediate molecule and can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other amino-protecting group(s). Preferred amino-protecting groups are *t*-butoxycarbonyl (*t*-Boc), allyloxycarbonyl and benzyloxycarbonyl (CbZ). Further examples of groups referred to by the above terms are described by J. W. Barton, "Protective Groups in Organic Chemistry", J. G. W. McOmie, Ed., Plenum Press, New York, N.Y., 1973, Chapter 2, and T. W. Greene, "Protective Groups in Organic Synthesis", John Wiley and sons, New York, N.Y., 1981, Chapter 7.

[0016] The term "hydroxy protecting group" refers to a substituent of an hydroxy group that is commonly employed to block or protect the hydroxy functionality while reactions are carried out on other functional groups on the compound. Examples of such hydroxy protecting groups include tetrahydropyranyl, 2-methoxyprop-2-yl, 1-ethoxyeth-1-yl, methoxymethyl, β -methoxyethoxymethyl, methylthiomethyl, *t*-butyl, *t*-amyl, trityl, 4-methoxytrityl, 4,4'-dimethoxytrityl, 4,4',4"-trimethoxytrityl, benzyl, allyl, trimethylsilyl, trimethylsilylethyl, (*t*-butyl)dimethylsilyl, and 2,2,2-trichloroethoxycarbonyl and the like. The species of hydroxy protecting group is not critical so long as the derivatized hydroxy group is stable to the conditions of the subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the molecule. A preferred hydroxy protecting group is trimethylsilylethyl. Further examples of hydroxy protecting groups are described in T.W. Greene, "Protective Groups in Organic Synthesis," John Wiley and Sons, New York, N.Y., (2nd ed., 1991) chapters 2 and 3. The term "protected hydroxy" refers to a hydroxy group bonded to one of the above hydroxy protecting groups.

[0017] The term "inhibiting", i.e. a method of inhibiting fungal activity, includes stopping, retarding or prophylactically hindering or preventing the growth or any attending characteristics and results from the existence of a fungus.

[0018] The term "contacting", i.e. contacting a compound of the invention with a fungus, includes a union or junction, or apparent touching or mutual tangency of a compound of the invention with a fungus. However, the term does not imply any further limitations to the process, such as by mechanism of inhibition, and the methods are defined to encompass the spirit of the invention, which is to inhibit fungal activity by the action of the compounds and their inherent antifungal properties, or in other words, the compounds, used in the claimed methods are the causative agent for such inhibition.

[0019] The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds of the above formula which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a mineral or organic acid or an inorganic base. Such salts are known as acid addition and base addition salts.

[0020] Acids commonly employed to form acid addition salts are mineral acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid and the like, and organic acids such as *p*-toluenesulfonic, methanesulfonic acid, oxalic acid, *p*-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propionate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, γ -hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulfonic acid.

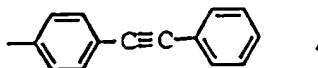
[0021] Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate,

sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred.

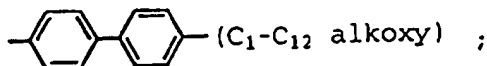
[0022] It should be recognized that the particular counterion forming a part of any salt of this invention is not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

[0023] Preferred compounds of this invention are those compounds of formula I where:

R³ is

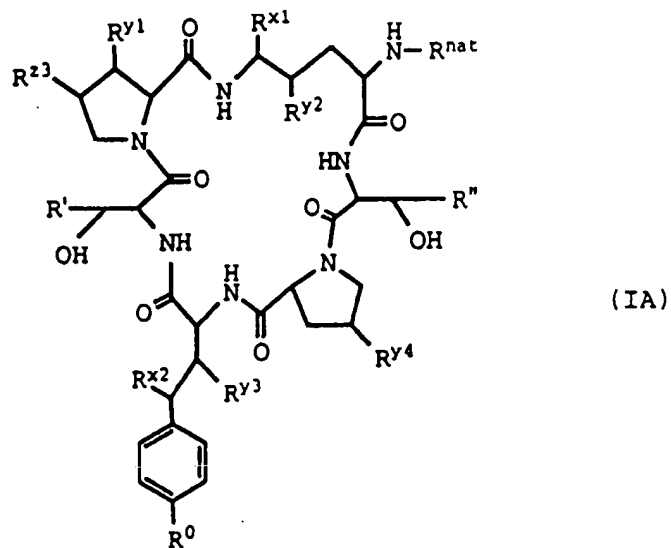


or



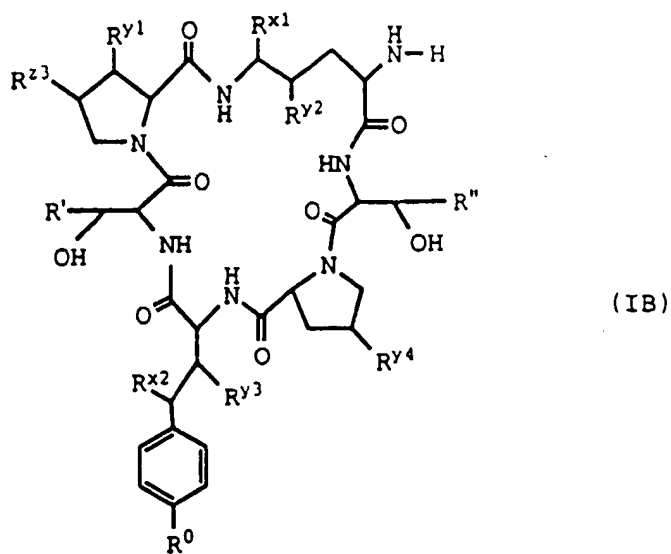
20 or a pharmaceutically acceptable salt thereof.

[0024] The compounds of formula I may be prepared according to Reaction Scheme I, as follows.

Reaction Scheme I

25

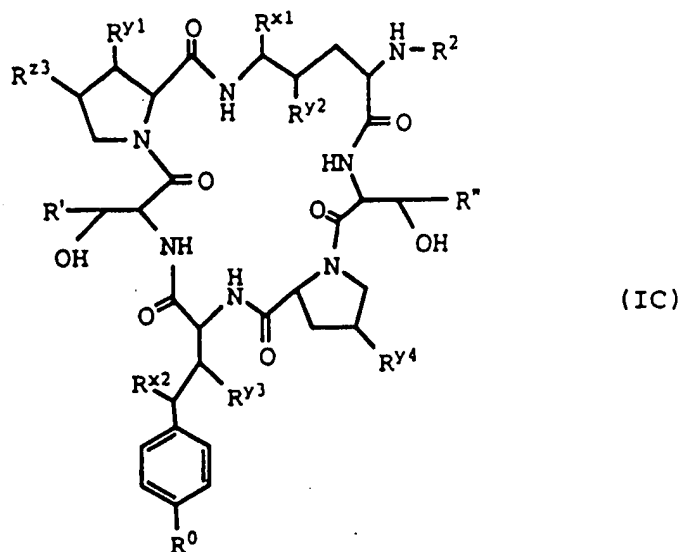
A. deacylate



50

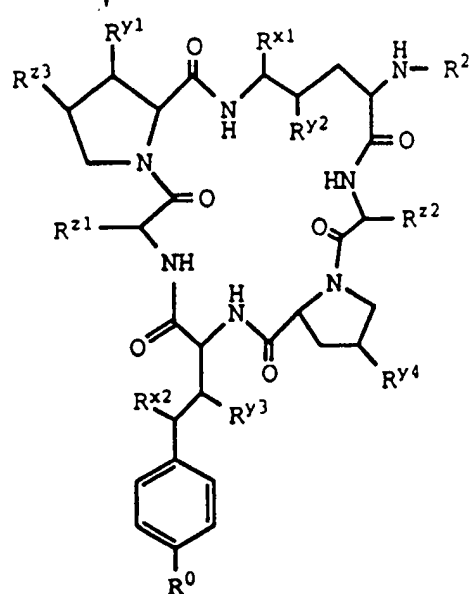
B. re-acylate

55



25

C. Retro-aldol
reaction



wherein:

50 R^{nat} is a naturally occurring cyclic peptide sidechain;
 R^1 is hydrogen, methyl or $-CH_2C(O)NH_2$;
 R^2 is hydrogen or methyl; and
 R^3 , R^z2 , R^z3 , R^x1 , R^x1' , R^x2 , R^y1 , R^y2 , R^y3 , R^y4 , R^0 and R^2 are as defined above.

55 **[0025]** Reaction scheme I, above, is accomplished by carrying out reactions A-C, above. Once a reaction is complete, the intermediate compound may be isolated by procedures well-known in the art, for example, the compound may be crystallized or precipitated and then collected by filtration, or the reaction solvent may be removed by extraction, evaporation or decantation. The intermediate compound may be further purified, if desired, by common techniques such

as crystallization or precipitation or chromatography over solid supports such as silica gel, alumina and the like, before carrying out the next step of the reaction scheme.

[0026] In reaction IA, a naturally occurring cyclic peptide of the formula IA is deacylated using procedures known in the art to provide an amino nucleus of formula IB. This reaction is typically carried out using enzymatic deacylation by exposing the naturally occurring cyclic peptide to a deacylase enzyme. The deacylase enzyme may be obtained from the microorganism *Actinoplanes utahensis* and used substantially as described in U.S. Patent Nos. 4,293,482 and 4,304,716, herein incorporated by reference. The deacylase enzyme may also be obtained from the *Pseudomonas* species. Deacylation may be accomplished using whole cells of *Actinoplanes utahensis* or *Pseudomonas* or the crude or purified enzyme thereof or using an immobilized form of the enzyme. See European Patent Application No. 0 460 882 (December 11, 1991). Examples of naturally occurring cyclic peptides which may be used as starting materials include aculeacin (palmitoyl side chain), tetrahydroechinocandin B (stearoyl side chain), mulundocandin (branched C₁₅ side chain), L-671,329 (C₁₆ branched side chain), S 31794/F1 (tetradecanoyl side chain), sporiofungin (C₁₅ branched side chain), FR901379 (palmitoyl side chain) and the like. A preferred naturally occurring cyclic peptide is echinocandin B (a compound of formula IA where R¹, R² and R³ are each methyl, R^{x1}, R^{x2}, R^{y1}, R^{y2}, R^{y3}, R^{y4} and R⁰ are each hydroxy and R² is linoleoyl).

[0027] In Reaction IB, the amino nucleus of formula IB is re-acylated using procedures known in the art to provide a compound of formula IC where R² is an acyl group as defined hereinabove.

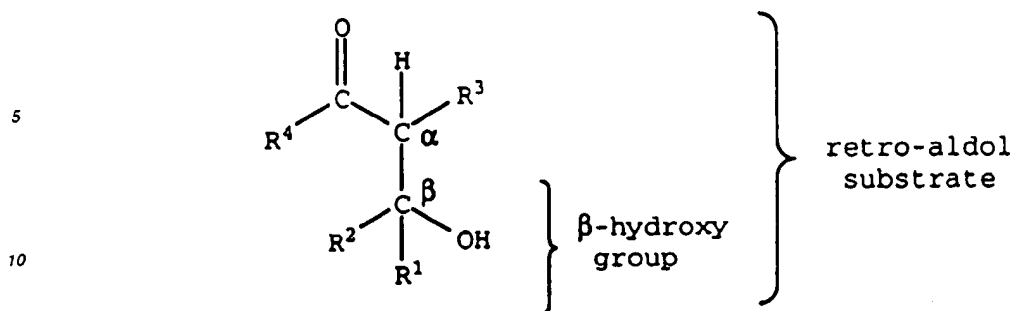
[0028] For example, the amino nucleus may be acylated by reaction with an appropriately substituted acyl halide, preferably in the presence of an acid scavenger such as a tertiary amine, such as triethylamine. The reaction is typically carried out at a temperature of from about -20°C to about 25°C. Typical solvents for this reaction include polar aprotic solvents such as dioxane or dimethylformamide. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction.

[0029] The amino nucleus may also be acylated by reaction with an appropriately substituted carboxylic acid, in the presence of a coupling agent. Typical coupling agents include dicyclohexylcarbodiimide (DCC), N,N'-carbonyldiimidazole, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and the like.

[0030] In addition, the amino nucleus may be acylated with an activated ester of a carboxylic acid such as an ester of a carboxylic acid of the formula R²-COOH and p-nitrophenyl, 2,4,5-trichlorophenyl, hydroxybenzotriazole hydrate (HOBT·H₂O), pentafluorophenol, N-hydroxysuccinimide and the like. Preferred acylating moieties are the active esters of the carboxylic acid R²-COOH such as 2,4,5-trichlorophenyl ester and benzotriazole ester. The reaction is typically carried out for one to sixty five hours at a temperature from about 0°C to about 30°C in an aprotic solvent. The reaction is generally complete after about twenty four to forty eight hours when carried out at a temperature of from about 15°C to about 30°C. Typical solvents for this reaction are tetrahydrofuran and dimethylformamide or a mixture of such solvents. The amino nucleus is generally employed in equimolar proportions relative to the activated ester or with a slight excess of the amino nucleus.

[0031] In Reaction IC, one or both of the β-hydroxy groups are selectively removed from a compound of formula IC to provide a compound of formula I. Specifically, the compound of formula IC is heated in an aprotic solvent to between about 50°C and 100°C in the presence of at least about five equivalents (preferably at least ten equivalents) of a retro-aldol-promoting reagent selected from the group consisting of trimethylamine-N-oxide, triethylamine-N-oxide, trimethylamine-N-oxide-hydrate and trimethylamine-N-oxide-hydrate. The reaction is generally conducted in a sealed tube or in a round bottom flask supplied with a water condenser for approximately three to seventy two hours. The reaction is preferably conducted at a temperature of about 70°C to about 100°C, preferably at about 70°C. Typical aprotic solvents include dimethylformamide, tetrahydrofuran, acetonitrile, dimethylsulfoxide and the like. A preferred solvent is acetonitrile. Preferred retro-aldol-promoting reagents are trimethylamine-N-oxide and trimethylamine-N-oxide-hydrate. The most preferred retro-aldol-promoting reagent is trimethylamine-N-oxide-hydrate. This reaction is preferably conducted after protecting the aminal hydroxy (R^{x1}) of the compound of formula IC. The hydroxy protecting group may be removed after removal of the β-hydroxy group(s), using procedures known in the art.

[0032] The term "aldol" indicates a molecule that is both an alcohol and an aldehyde or ketone wherein the hydroxyl and carbonyl functional groups are on adjacent carbon atoms as shown below. Thus, as used herein, the term "β-hydroxy group" refers to the alcohol moiety including both the β-carbon and the hydroxyl functional group that is resident on the β-carbon according to the following general structure:



15 where:

R¹ and R² are independently hydrogen or methyl;

R³ and R⁴ taken together with the atoms to which they are attached form a cyclic peptide.

20 [0033] The compounds where R^{x1} is -O-R^{x1'} may be prepared by the corresponding compound where R^{x1} is hydroxy with an appropriately substituted alcohol in the presence of an acid to provide a compound of formula I wherein R^{x1} is -O-R^{x1'} where R^{x1'} is C₁-C₆ alkyl, benzyl, -(CH₂)₂Si(CH₃)₃, -CH₂CH=CH₂, -(CH₂)_aCOOH, -(CH₂)_bNR^{w1}R^{w2}, -(CH₂)_cPOR^{w3}R^{w4} or -[(CH₂)₂O]_d-(C₁-C₆)alkyl. The compounds where R^{x1} is -O-R^{x1'} are preferably prepared after removal of the β-hydroxy group(s). The reaction is typically carried out in a polar aprotic solvent such as dioxane or dimethylsulfoxide at a temperature of from about 0°C to about 35°C, preferably at about room temperature. Solvent

25 choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction. Preferred acids include p-toluenesulfonic acid, hydrochloric acid and camphorsulfonic acid.

[0034] The compounds where R^{x1} is -(CH₂)_bNR^{w1}R^{w2} where R^{w1} and R^{w2} are hydrogen may be prepared via a protected compound wherein R^{x1} is -(CH₂)_bNHR^a where R^a is an amino protecting group. The resultant protected

30 compound may be deprotected according to procedures known in the art.

[0035] The compounds where R^{x1} is -CH₂CHOHCH₂OH may be prepared by hydroxylating a compound of formula I where R^{x1} is -CH₂CH=CH₂ with osmium tetroxide in the presence of a catalyst at a temperature in the range of from about 0°C to about 40°C for about one to twenty four hours in a organic/aqueous solvent mixture, for example dioxane/

35 water. Suitable catalysts include N-methylmorpholine N-oxide (NMO) and the like. Typical solvents suitable for use in this reaction include dimethylformamide, tetrahydrofuran, ethyl acetate, dioxane or a mixture of these solvents. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction. The reaction is preferably conducted at a temperature in the range of from about 20°C to about 30°C for about eighteen to twenty four hours.

[0036] The compounds where R⁰ is hydroxy may be phosphorylated by reaction with an appropriately substituted alkyl, phenyl or benzyl phosphate to provide a compound where R⁰ is -O-P(O)OH-OR¹ where R¹ is C₁-C₆ alkyl, phenyl or benzyl, or by reaction with an appropriately substituted alkyl, phenyl or benzyl phosphonic acid to provide a compound where R⁰ is -O-P(O)OH-R¹ where R¹ is C₁-C₆ alkyl, phenyl or benzyl, to provide a compound of formula I where R⁰ is a group of the formula -OP(O)OH-OR¹ or -OP(O)OH-R¹. This reaction is preferably conducted after removal of the β-hydroxy group(s). The phosphonic acid is typically used in an activated form, for example as a phosphonic halide,

45 preferably a phosphonic chloride. The reaction is carried out in the presence of a base such as lithium trimethylsilanolate (LiOTMS), lithium bis(trimethylsilyl)amide (LHMDS), pyridine and the like. The reaction is typically carried out for up to one hour at a temperature from about -30°C to about 0°C in an aprotic solvent such as tetrahydrofuran and dimethylformamide. The reaction is generally complete in about fifteen minutes when carried out under these conditions. The phosphate or phosphonate reactant is generally employed in equimolar proportions to about a one mole excess relative

50 to the amino nucleus in the presence of an equimolar or slight excess of the base. Phosphorylation of an amino nucleus with unprotected aminal hydroxy groups is typically carried out at lower temperatures, for example from about -30°C to about -15°C.

[0037] Alternatively, the compounds where R^{x1} is hydroxy may be phosphorylated after first protecting the hydroxy moiety with an hydroxy protecting group using procedures known in the art. For example, the reaction is typically carried

55 out by combining the compound with a suitable hydroxy protecting group in the presence of a catalyst at a temperature in the range of from about 0°C to about 40°C for about one to five hours in a mutual inert solvent. The hydroxy protecting group is generally employed in an amount ranging from about equimolar proportions to about a 100 molar excess relative to the compound, preferably in a large molar excess. Suitable catalysts include strong acids such as p-tolue-

nesulfonic acid, camphorsulfonic acid (CSA), hydrochloric acid, sulfuric acid, trifluoroacetic acid and the like. Typical solvents suitable for use in this reaction include any organic solvent such as dioxane. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction. The reaction is preferably conducted at a temperature in the range of from about 20°C to about 30°C for about two to four hours. The protected compound is then phosphorylated as described above. The hydroxy protecting group is then removed according to procedures known in the art to provide the desired phosphorylated compound. For example, the protecting groups can be removed by reaction with a Lewis acid in a mutual inert organic solvent such as methylene chloride. Examples of Lewis acids include trimethylsilylbromide, boron trifluoride etherate and the like. The reaction is typically carried out at a temperature of from about 0°C to about 40°C, preferably at a temperature of from about 20°C to about 30°C. A preferred Lewis acid is boron trifluoride etherate.

[0038] The dideoxy compounds are prepared by removing the benzylic and aminor hydroxy groups (R^{x2} and R^{x1} , respectively). The hydroxy groups may be removed by subjecting a non-dideoxy compound to a strong acid and a reducing agent at a temperature of between -5°C and 70°C, in a suitable solvent. Typical strong acids include trichloroacetic acid, trifluoroacetic acid or borontrifluoride etherate. A preferred strong acid is trifluoroacetic acid. Typical reducing agents include sodium cyanoborohydride or triethylsilane. A preferred reducing agent is triethylsilane. Suitable solvents include methylene chloride, chloroform or acetic acid, preferably methylene chloride. The strong acid should be present in an amount of from 2 to 80 mol per mol of substrate, and the reducing agent should be present in an amount of 2 to 80 mol per mol of substrate. This process affords selective removal of the aminor and benzylic hydroxy groups.

[0039] The cyclic peptides used to make the compounds of the present invention may be prepared by fermentation of known microorganisms. For example, the cyclic peptide of formula IB where R^1 , R^* and R^{z3} are methyl, and R^{x1} , R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 are each hydroxy (cyclic nucleus corresponding to A-30912A) may be prepared using the procedure detailed in Abbott et al., U.S. Pat. Ser. No. 4,293,482, which is herein incorporated by reference. The cyclic peptide of formula IB where R^1 , R^* and R^{z3} are methyl, R^{x1} is hydroxy, R^{x2} is hydrogen, and R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 are each hydroxy (cyclic nucleus corresponding to A-30912B) may be prepared using the procedure detailed in Abbott et al., U.S. Pat. Ser. No. 4,299,763, which is herein incorporated by reference. Aculeacin may be prepared using the procedure detailed in Mizuno et al., U.S. Pat. Ser. No. 3,978,210 which is herein incorporated by reference. The cyclic peptide of formula IB where R^1 is $-CH_2C(O)NH_2$, R^* is methyl, R^{z3} is hydrogen, and R^{x1} , R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} and R^0 are each hydroxy may be prepared by deacylating the cyclic peptide prepared using the procedure detailed in Chen et al., U.S. Pat. Ser. No. 5,198,421, which is herein incorporated by reference.

[0040] The R^2 -COOH precursor acids, used to acylate the amino nucleus of formula IB, may be obtained commercially or prepared according to procedures known in the art. For example, an appropriately substituted phenyl boronic acid or biphenyl boronic acid reactant may be reacted with a p-halobenzoic acid reactant in the presence of a catalyst such as tetrakis(triphenylphosphine)palladium and an inorganic base such as potassium carbonate in a mutual inert organic solvent such as toluene at a temperature of from about 20°C to the reflux temperature of the reaction mixture to provide the corresponding biphenyl carboxylic acids and terphenyl carboxylic acids used to prepare the compounds of formula I. The reaction is typically carried out with equimolar proportions of the boronic acid reactant and the p-benzoic acid reactant, or a slight molar excess of the p-benzoic acid reactant relative to the boronic acid reactant, and a 1-2 molar excess of the inorganic base. The reaction is generally complete after about four to about ten hours when carried out at reflux temperature in toluene.

[0041] The boronic acid reactant may be prepared by reacting an appropriately substituted halophenyl or halobiphenyl reactant with two equivalents of triisopropyl borate in the presence of an alkyl lithium, for example sec-butyl lithium, in a mutual inert solvent such as tetrahydrofuran. The alkyl lithium is typically employed in a slight molar excess relative to the halophenyl or halobiphenyl reactant. The alkyl lithium is typically combined with the solvent by dropwise addition at reduced temperatures (<-70°C) and allowed to stir for approximately thirty minutes before the addition of the triisopropyl borate. The reaction is typically carried out initially at a temperature of from about -100°C to about -50°C, preferably from about -75°C to about -85°C for thirty minutes to two hours and then warmed to room temperature and reacted for an additional one to three hours. The reaction is generally complete in from several minutes to about four hours. When the reaction is substantially complete, the boronic acid moiety is formed by the addition of an acid. A preferred acid is a 1N hydrochloric acid solution.

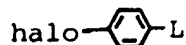
[0042] The carboxylic acids, R^2 -COOH, having an acetylene moiety may be prepared by reacting an appropriately substituted acetylene reactant with an appropriately substituted phenyl or biphenyl reactant of the formula



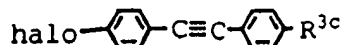
where L is a suitable leaving group such as bromo, iodo, methanesulfonate, toluenesulfonate, trifluoromethanesul-

ionate and the like, in the presence of a catalyst and preferably in the presence of an acid scavenger in a mutual inert solvent such as acetonitrile. Examples of acid scavengers include triethylamine and pyridine, preferably triethylamine. A preferred catalyst is formed *in situ* from palladium (II) chloride, triphenylphosphine and copper (I) iodide. The reaction is typically carried out for thirty minutes to twenty one hours at a temperature from about room temperature to the reflux temperature of reaction mixture. The reaction is generally complete after about two to about six hours when carried out at reflux temperature.

[0043] Alternatively, a suitably substituted phenyl reactant of the formula



may be reacted with an appropriately substituted acetylene reactant as described above to provide, for example, a compound of the formula



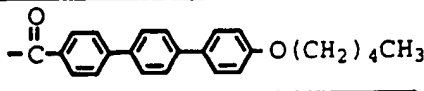
which can be coupled with a phenyl boronic acid reactant as described above.

[0044] The following Preparations and Examples further describe how to synthesize the compounds of the present invention. The terms melting point, proton nuclear magnetic resonance spectra, mass spectra, infrared spectra, ultra-violet spectra, elemental analysis, high performance liquid chromatography, and thin layer chromatography are abbreviated "m.p.", "NMR", "MS", "IR", "UV", "Analysis", "HPLC", and "TLC", respectively. In addition, the absorption maxima listed for the IR spectra are only those of interest and not all of the maxima observed.

Comparative Example 1

[0045]

A. Preparation of the compound of formula IC where R', R" and R²³ are each methyl, R^{x1} and R^{x2} are each hydroxy, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is

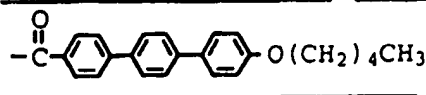


[0046] The N-acyl cyclic peptide derivatives listed in Table 3, below were prepared by dissolving 348.1 g (60.2 mmol) of the A30912A nucleus (compound of formula IB where R', R" and R²³ are each methyl, R^{x1} and R^{x2} are each hydroxy, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, and R⁰ is hydroxy) and 26.0 g (48.2 mmol) of the 2,4,5-trichlorophenol ester of [[[(4"-pentyloxy)-1,1':4',1"-terphenyl]-4-carboxylic acid, in 8.5 l of dimethylformamide. The resultant reaction mixture was stirred for approximately 48 hours at room temperature and then the solvent was removed *in vacuo* to provide a residue. This residue was slurried in ether, collected by filtration, washed with methylene chloride and then dissolved in methanol or a 1:1 (v/v) acetonitrile/water mixture. The resultant solution is subjected to reverse phase HPLC (C18; eluent of 20-40% aqueous acetonitrile containing 0.5% monobasic ammonium phosphate (w/v); 20 ml/min.; 230 nm). After removing the unreacted A30912A nucleus, the desired product was eluted from the column using an eluent of aqueous acetonitrile. The fractions containing the desired product are combined and then reduced to dryness *in vacuo* to provide the desired acylated nucleus.

Yield: 18 g.

MS(FAB): : 1140.5103 (M⁺).

B. Preparation of the compound of formula IC where R¹, R² and R²³ are each methyl, R^{x1} and R^{x2} are each hydrogen, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is



[0047] To a mixture of 5 g (4.4 mmol) of the compound prepared in Example 1A and 17 ml of trifluoroacetic acid in 250 ml of methylene chloride, was added 35 ml of triethylsilane. When the reaction was substantially complete, as indicated by HPLC (C18, eluent of 55% acetonitrile; 2 ml/min; 280 nm; R_T (starting material) = 4.19 min.; R_T (product) = 6.40 min.), the reaction mixture was concentrated *in vacuo* to provide a solid. This solid was slurried in 100 ml of 50% aqueous acetone and then dissolved by adjusting the pH of the mixture to approximately pH 7. The resultant solution was poured into a large volume of water (approximately 1 liter) resulting in the precipitation of a white solid. This solid was isolated by filtration through a sintered glass funnel, washed with diethyl ether and then dried *in vacuo* at 55°C to provide 3.718 g of the titled compound. The funnel was washed with methanol to collect the remaining solid, which was dried *in vacuo* to provide an additional 0.154 g of the titled compound.

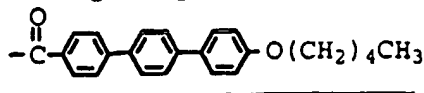
Yield: 3.872 g (79%).

MS (FAB): m/e 1108.7 (M)

HPLC: (eluent of 55% acetonitrile; 2 ml/min.; 280 nm):

R_T = 6.43 min.

C. Preparation of the compound of formula I where R^{z1} and R^{z2} are each hydrogen, R^{z3} is methyl, R^{x1} and R^{x2} are each hydrogen, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is

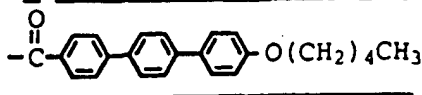


[0048] The compound prepared in Example 1B was dissolved in a 1:1 mixture of acetonitrile and dimethylformamide. To this solution, was added TNO-hydrate (1.6 g, 14.3 mmol) all at once. The reaction mixture was heated at 100°C for 48 hours after which the mixture was cooled to room temperature and concentrated to approximately one-half its original volume. The crude residue was dissolved with acetic acid and purified by reverse phase preparative HPLC to yield 150 mg final product (52%). TNO-hydrate converted the two threonine residues into glycine residues and left the homotyrosine untouched as confirmed by fast atom bombardment mass spectrometry, which confirmed the molecular weight of the product having the chemical formula C₅₄H₆₅N₇O₁₃; calculated - 1020.1; found - 1020.8. (M+H).

Comparative Example 2

[0049]

Alternative Preparation of the compound of formula I where R^{z1} and R^{z2} are each hydrogen, R^{z3} is methyl, R^{x1} and R^{x2} are each hydrogen, R^{y1}, R^{y2}, R^{y3} and R^{y4} are each hydroxy, R⁰ is hydroxy and R² is



[0050] The compound prepared in Example 1B was dissolved in 20 ml of a 1:1 mixture of acetonitrile and dimethyl-

formamide. To this solution, was added trimethylamine N-oxide dihydrate (2.51 g, 22.6 mmol) all at once. The reaction mixture was heated at 100°C for 24 hours in a sealed tube after which the mixture was cooled to room temperature and concentrated *in vacuo* to approximately one-half its original volume. The crude residue was isolated by filtration and then rinsed with cold dimethylformamide. The resultant filtrate was dried *in vacuo* to provide a residue. This residue was purified using reverse phase HPLC (3x40x10 radial column, eluent of 50% acetonitrile in water, 60 ml/min., 280 nm) to yield 330 mg final product (72%).

Fast atom bombardment mass spectrometry confirmed the molecular weight of the product having the chemical formula $C_{54}H_{65}N_7O_{13}$; calculated - 1020.1; found - 1020.4 (M+H).

[0051] The compounds of formula I exhibit antifungal activity. For example, the compounds of formula I inhibit the growth of various infectious fungi including *Candida spp.* such as *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, or *C. tropicalis*, *C. lusitaniae*; *Torulopus spp.* such as *T. glabrata*; *Aspergillus spp.* such as *A. fumigatus*; *Histoplasma spp.* such as *H. capsulatum*; *Cryptococcus spp.* such as *C. neoformans*; *Blastomyces spp.* such as *B. dermatitidis*; *Fusarium spp.*, *Trichophyton spp.*, *Pseudallescheria boydii*, *Coccidioides immitis*, *Sporothrix schenckii* and the like.

[0052] Antifungal activity of a test compound is determined *in vitro* by obtaining the minimum inhibitory concentration (MIC) of the compound using a standard agar dilution test or a disc-diffusion test. The compound is then tested *in vivo* (in mice) to determine the effective dose of the test compound for controlling a systemic fungal infection.

[0053] Accordingly, the compound prepared in Example 1C was tested for antifungal activity against *C. albicans*. The minimal inhibitory concentration (MIC) of this compound against *C. albicans* was 0.312 (µg/ml).

[0054] In addition, the effective dose of the compound prepared in Example 1C, for controlling a systemic fungal infection (*C. albicans*), was tested *in vivo* (mice). The ED₅₀ for this compound was >2.5.

[0055] The compounds of formula I are active *in vitro* and *in vivo* and are useful in combating either systemic fungal infections or fungal skin infections. Accordingly, the present invention provides a method of inhibiting fungal activity comprising contacting a compound of formula I, or a pharmaceutically acceptable salt thereof, with a fungus. A preferred method includes inhibiting *Candida albicans* or *Aspergillus fumigatus* activity. The present invention further provides a method of treating a fungal infection which comprises administering an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof, to a host in need of such treatment. A preferred method includes treating a *Candida albicans* or *Aspergillus fumigatus* infection.

[0056] With respect to antifungal activity, the term "effective amount," means an amount of a compound of the present invention which is capable of inhibiting fungal activity. The dose administered will vary depending on such factors as the nature and severity of the infection, the age and general health of the host and the tolerance of the host to the antifungal agent. The particular dose regimen likewise may vary according to such factors and may be given in a single daily dose or in multiple doses during the day. The regimen may last from about 2-3 days to about 2-3 weeks or longer. A typical daily dose (administered in single or divided doses) will contain a dosage level of from about 0.01 mg/kg to about 100 mg/kg of body weight of an active compound of this invention. Preferred daily doses generally will be from about 0.1 mg/kg to about 60 mg/kg and ideally from about 2.5 mg/kg to about 40 mg/kg.

[0057] The present invention also provides pharmaceutical formulations useful for administering the antifungal compounds of the invention. Accordingly, the present invention also provides a pharmaceutical formulation comprising one or more pharmaceutically acceptable carriers, diluents or excipients and a compound of claim 1. The active ingredient in such formulations comprises from 0.1% to 99.9% by weight of the formulation, more generally from about 10% to about 30% by weight. By "pharmaceutically acceptable" it is meant that the carrier, diluent or excipient is compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0058] A compound of formula I may be administered parenterally, for example using intramuscular, subcutaneous, or intra-peritoneal injection, nasal or oral means. In addition to these methods of administration, a compound of formula I may be applied topically for skin infections.

[0059] For parenteral administration the formulation comprises a compound of formula I and a physiologically acceptable diluent such as deionized water, physiological saline, 5% dextrose and other commonly used diluents. The formulation may contain a solubilizing agent such as a polyethylene glycol or polypropylene glycol or other known solubilizing agent. Such formulations may be made up in sterile vials containing the antifungal and excipient in a dry powder or lyophilized powder form. Prior to use, a physiologically acceptable diluent is added and the solution withdrawn via syringe for administration to the patient.

[0060] The present pharmaceutical formulations are prepared by known procedures using known and readily available ingredients. In making the compositions of the present invention, the active ingredient will generally be admixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders and the like.

[0061] For oral administration, the antifungal compound is filled into gelatin capsules or formed into tablets. Such tablets may also contain a binding agent, a dispersant or other suitable excipients suitable for preparing a proper size tablet for the dosage and particular antifungal compound of the formula I. For pediatric or geriatric use the antifungal compound may be formulated into a flavored liquid suspension, solution or emulsion. A preferred oral formulation is linoleic acid, cremophor RH-60 and water and preferably in the amount (by volume) of 8% linoleic acid, 5% cremophor RH-60, 87% sterile water and a compound of formula I in an amount of from about 2.5 to about 40 mg/ml.

[0062] For topical use the antifungal compound may be formulated with a dry powder for application to the skin surface or it may be formulated in a liquid formulation comprising a solubilizing aqueous liquid or non-aqueous liquid, e.g., an alcohol or glycol.

[0063] The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way. The term "active ingredient" means a compound according to formula I or a pharmaceutically acceptable salt thereof.

Formulation 1

[0064] Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active ingredient	250
Starch, dried	200
Magnesium stearate	10
Total	460 mg

Formulation 2

[0065] A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665 mg

[0066] The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

[0067] An aerosol solution is prepared containing the following components:

	Weight
Active ingredient	0.25
Methanol	25.75
Propellant 22 (Chlorodifluoromethane)	74.00
Total	100.00

[0068] The active compound is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

[0069] Tablets, each containing 60 mg of active ingredient, are made as follows:

EP 0 757 058 B1

Active ingredient	60 mg
Starch	45 mg
Microcrystalline cellulose	35 mg
Polyvinylpyrrolidone (as 10% solution in water)	4 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1 mg
Total	150 mg

[0070] The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The aqueous solution containing polyvinyl-pyrrolidone is mixed with the resultant powder, and the mixture then is passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

[0071] Capsules, each containing 80 mg of active ingredient, are made as follows:

Active ingredient	80 mg
Starch	59 mg
Microcrystalline cellulose	59 mg
Magnesium stearate	2 mg
Total	200 mg

[0072] The active ingredient, cellulose, starch and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

[0073] Suppositories, each containing 225 mg of active ingredient, are made as follows:

Active ingredient	225 mg
Saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

[0074] The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

Formulation 7

[0075] Suspensions, each containing 50 mg of active ingredient per 5 ml dose, are made as follows:

Active ingredient	50 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 ml
Benzoic acid solution	0.10 ml
Flavor	q.v.
Color	q.v.
Purified water to total	5 ml

[0076] The active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with a portion of the water and added, with stirring. Sufficient water is then added to produce the required volume.

5 Formulation 8

[0077] An intravenous formulation may be prepared as follows:

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Active ingredient	100 mg
Isotonic saline	1,000 ml

[0078] Active ingredient 100 mg Isotonic saline The solution of the above ingredients generally is administered intravenously to a subject at a rate of 1 ml per minute.

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Claims

1. A compound of formula I:

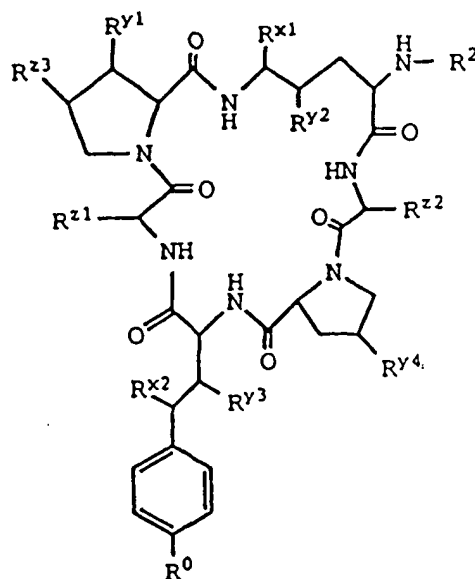
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wherein:

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R^{z1} and R^{z2} are both hydrogen;

R^{z3} is hydrogen or methyl;

R^{x1} is hydrogen;

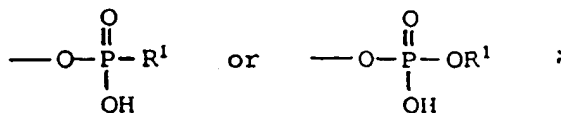
R^{x2} is hydrogen;

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R^{y1} , R^{y2} , R^{y3} and R^{y4} are independently hydroxy or hydrogen;

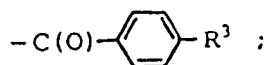
R^0 is hydroxy, $-OP(O)(OH)_2$ or a group of the formulae:

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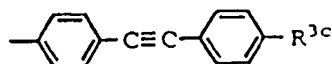
R₁ is C₁-C₆ alkyl, phenyl, p-halo-phenyl, p-nitrophenyl, benzyl, p-halo-benzyl or p-nitro-benzyl;
R₂ is

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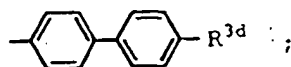
R³ is

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or

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R^{3c} and R^{3d} are independently hydrogen, C₁-C₁₂ alkyl, C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy or -O-(CH₂)₂-O-(C₁-C₁₂ alkyl);

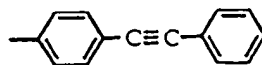
or a pharmaceutically acceptable salt thereof.

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2. A compound according to claim 1 where:

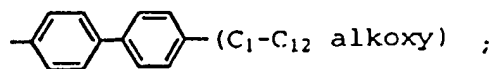
R³ is

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or

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or a pharmaceutically acceptable salt thereof.

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3. A pharmaceutical formulation comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any one of the claims 1 to 2, associated with one or more pharmaceutically acceptable carriers, diluents or excipients therefore.

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4. A compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 2, for use as a pharmaceutical.

Patentansprüche

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1. Verbindung der Formel I

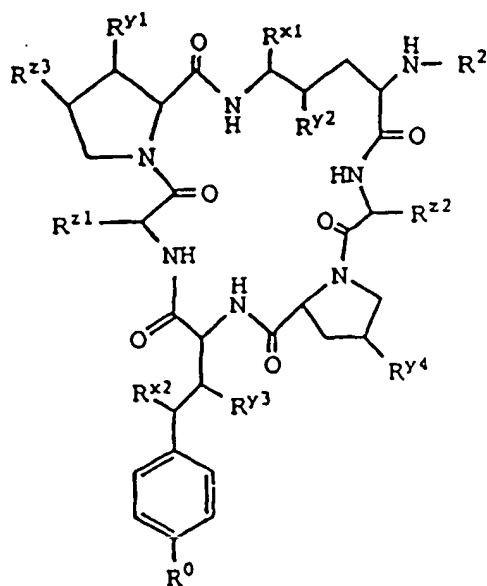
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I

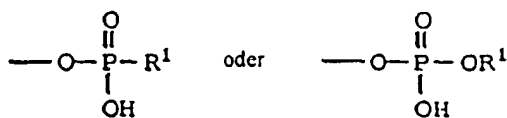
worin

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R^{z1} und R^{z2} beide für Wasserstoff stehen,
 R^{z3} für Wasserstoff oder Methyl steht,
 R^{x1} für Wasserstoff steht,
 R^{x2} für Wasserstoff steht,
 R^{y1} , R^{y2} , R^{y3} und R^{y4} jeweils unabhängig für Hydroxy oder Wasserstoff stehen,
 R^0 für Hydroxy, $-OP(O)(OH)_2$ oder eine Gruppe der folgenden Formeln steht

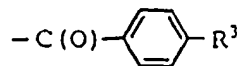
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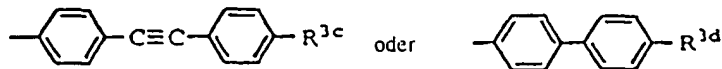
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R^1 für C_1 - C_6 Alkyl, Phenyl, p-Halogenphenyl, p-Nitrophenyl, Benzyl, p-Halogenbenzyl oder p-Nitrobenzyl steht,
 R^2 steht für

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 R^3 steht für

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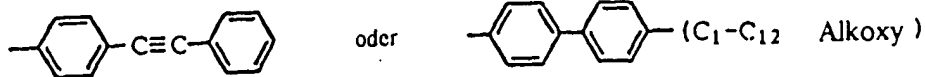


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R^{3c} und R^{3d} unabhängig für Wasserstoff, C_1 - C_{12} Alkyl, C_2 - C_{12} Alkynyl, C_1 - C_{12} Alkoxy oder $-O-(CH_2)_2-O-(C_1-C_{12}$ Alkyl) stehen,

oder ein pharmazeutisch annehmbares Salz hiervon.

2. Verbindung nach Anspruch 1, worin R^3 steht für

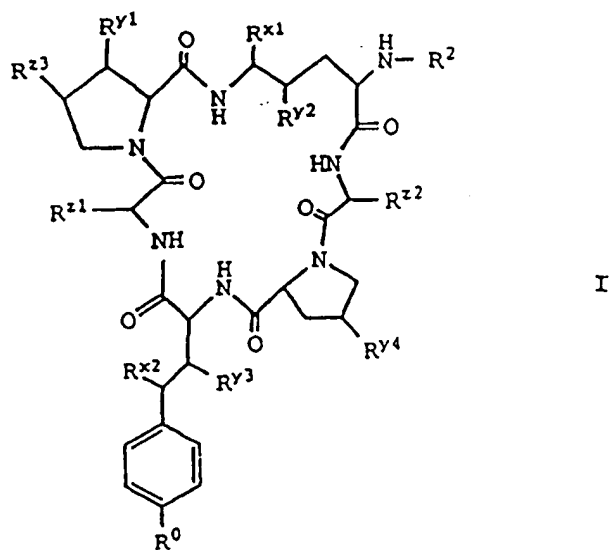


3. Pharmazeutische Formulierung, die eine Verbindung der Formel I oder ein pharmazeutisch annehmbares Salz hiervon nach einem der Ansprüche 1 bis 2 zusammen mit einem oder mehreren pharmazeutisch annehmbaren Trägern, Verdünnungsmitteln oder Hilfsstoffen hierfür enthält.

4. Verbindung der Formel I oder ein pharmazeutisch annehmbares Salz hiervon nach einem der Ansprüche 1 bis 2 zur Verwendung als Pharmazeutikum.

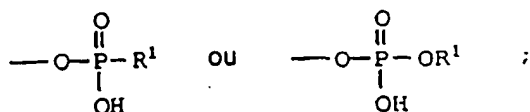
20 Revendications

1. Composé répondant à la formule I:

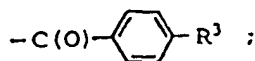


dans laquelle:

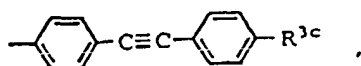
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- R^{z1} et R^{z2} représentent tous deux un atome d'hydrogène;
 R^{z3} représente un atome d'hydrogène ou un groupe méthyle;
 R^{x1} représente un atome d'hydrogène;
 R^{x2} représente un atome d'hydrogène;
 R^{y1} , R^{y2} , R^{y3} et R^{y4} représentent, indépendamment l'un de l'autre, un groupe hydroxyle ou un atome d'hydrogène;
 R^0 représente un groupe hydroxyle, un groupe $-OP(O)(OH)_2$ ou un groupe répondant aux formules :
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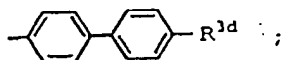
R¹ représente un groupe alkyle en C₁-C₆, un groupe phényle, un groupe p-halogéno-phényle, un groupe p-nitrophényle, un groupe benzyle, un groupe p-halogéno-benzyle ou un groupe p-nitro-benzyle;
R² représente



R³ représente



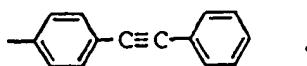
ou



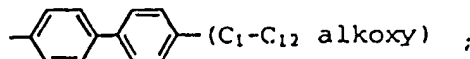
R^{3c} et R^{3d} représentent, indépendamment l'un de l'autre, un atome d'hydrogène, un groupe alkyle en C₁-C₁₂, un groupe alcynyle en C₂-C₁₂, un groupe alcoxy en C₁-C₁₂ ou un groupe -O-(CH₂)₂-O-alkyle en C₁-C₁₂;

ou un de ses sels pharmaceutiquement acceptables.

2. Composé selon la revendication 1, dans lequel:
R³ représente



ou



ou un de ses sels pharmaceutiquement acceptables.

3. Formulation pharmaceutique comprenant un composé répondant à la formule I ou un de ses sels pharmaceutiquement acceptables, selon l'une quelconque des revendications 1 à 2, en association avec un ou plusieurs supports, diluants ou excipients pharmaceutiquement acceptables pour le composé.
4. Composé répondant à la formule I ou un de ses sels pharmaceutiquement acceptables, selon l'une quelconque des revendications 1 à 2, à utiliser comme produit pharmaceutique.